



Serum S100B as a Predictor of Neuropsychological Outcomes
following Traumatic Brain Injury

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Declaration of Originality

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The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.



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Declaration of Interests

No competing or conflicting interests need to be declared for the studies contained in this thesis. This research received no financial payments from DiaSorin S.p.A, the copyright owner and distributor of the LIAISON[®] S100B assay. All materials were purchased independently using grant funding.



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Abstract

Empirically, depth of coma and duration of post-traumatic amnesia offer insufficient prognostic accuracy for neuropsychological outcomes following TBI. Recently, serum S100B has been proposed in the associated literature as a potential prognostic biomarker for outcome following TBI. Unfortunately, most of the studies investigating S100B have utilised crude outcome measures or mortality as the dependent variable in research design. This study aimed to investigate the relationship between S100B levels and existing TBI diagnostic measures, and to quantify its ability to predict future cognitive impairment, post-concussion syndrome symptomatology, and quality of life.

127 participants who presented to the Royal Hobart Hospital following a TBI were recruited for this longitudinal study. On presentation, serum samples were collected, freeze-stored, and then batch analysed for acute S100B levels. Participants completed a cognitive battery two months post injury, and then completed the British Columbia Post-Concussion Inventory and the Quality of Life Inventory six months post injury.

S100B levels were significantly correlated with depth of coma and duration of post-traumatic amnesia, and regression analyses showed that duration of post-traumatic amnesia could be predicted accurately by using S100B. Serum S100B concentrations accounted for a significant proportion of variance in various symptoms of post-concussion syndrome and poorer quality of life – however, S100B in isolation offers insufficient prognostic accuracy for these clinical outcomes. Unfortunately, however, S100B was not able to predict future cognitive impairment, suggesting that cognitive prognoses based on biological factors alone while in an acute setting remain elusive, if not illusive.

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Chapter One

Overview of the Thesis

Traumatic brain injury (TBI) refers to permanent or transient neurological dysfunction that has resulted from an external force to the brain. It is one of the most common neurological conditions, and unfortunately, the mortality and chronic disability that can result from TBI has improved little in the last 20 years (Masson et al., 2001). TBI can cause debilitating impairments to a person's physical, cognitive, behavioural and social functioning (Hammond et al., 2004). Although it is generally accepted that the long-term prognosis for mild and moderate cognitive recovery following TBI is good, it is also the case that a proportion of these patients will experience limited and incomplete recoveries (McAllister et al., 2006).

TBI is nearly always associated with cognitive deficits, but the extent of these deficits is considered to be highly variable (Isoniemi, Tenovuo, Portin, Himanend & Kairisto, 2006). The most commonly reported cognitive impairments that follow TBI occur in the domains of short-term memory, attention, executive functioning and speed of information processing; and these impairments have been well documented in the associated literature (Binder, Rohling & Larrabee, 1997; Dockree et al., 2006; Gentilini, Nichelli & Schoenhuber, 1989; Levin, High & Ewing-Cobbs, 1988; McAllister et al., 2006; Rapoport et al., 2005; Stuss et al., 1989).

Beers et al. (2007) have stated that increasing the accuracy of prediction of neuropsychological outcomes following TBI is essential. The authors argue that prognoses based on more reliable prediction methods can provide patients and their carers with accurate information and realistic expectations for outcome, and facilitate effective and appropriate referrals to rehabilitation services. Further, de Krujik et al. (2002) suggest that time and expenses that are invested in follow-up sessions with

specialists would be drastically reduced if there was a reliable means to identify those who are most expected to exhibit persistent symptoms while they are still in the context of an acute presentation.

TBI is regularly contextualised according to the “primary” and “secondary” components of the injury. The primary insult in TBI is most often the focal damage caused by the contact injury and the resulting diffuse axonal damage (Feala et al., 2013). Secondary injuries occur in the minutes to hours following the primary injury, involving alterations and interactions in cerebral blood flow and deranged biochemical metabolism. It is recognised that these secondary injuries significantly contribute to poor outcomes following TBI, and as such, the management of TBI patients is primarily targeted towards treatment of these secondary pathologies (Stein et al., 2012). It is argued that a better understanding of the cellular and molecular mechanisms implicated in pathophysiological changes following TBI is necessary in order to facilitate more accurate prognoses for functional outcomes (Böhmer et al., 2011).

Clinically, classifications of mild, moderate or severe TBI are dependent on the patient’s depth of coma, duration of post-traumatic amnesia, and duration of loss of consciousness (Begaz et al., 2006; Muñoz-Cespedes, Rios-Lago, Paul & Maetsu, 2005). Unfortunately, these measures when used to guide diagnostic evaluation are limited in their prognostic power.

Due to TBI causing diffuse axonal and small vessel injury that is often undetectable by computerised tomography (CT), emerging research has begun to investigate the relationship between brain-related proteins and TBI outcome (Vos & Verbeek, 2002; Begaz, Kyriacou, Segal, & Bazarian 2006). At a cellular level following TBI, the blood brain barrier is known to be disrupted and permeated, thus

allowing for brain specific molecules to enter the peripheral system. An indicator of blood brain barrier disruption would be beneficial to the TBI prognostic literature in order to guide clinical management and prognoses for these patients.

In medical sciences, biomarkers are used to provide diagnostic or prognostic information towards an altered physiological condition or disease state (Gonçalves, Leite & Nardin, 2008). Biomarkers can be detected and monitored in a variety of bodily fluids and tissues, and they may be detected in the human system by specifically contrasted imaging techniques, or alternatively, through laboratory analysis of the specific fluid or tissue (Jeter et al., 2013). Without exception, biomarker research has been the largest growing area in the domain of TBI prognostic literature.

The current direction of TBI biomarker research has focussed towards the investigation of the S100 calcium binding protein B (S100B) as it has been shown to fulfil the criteria of an ideal brain damage biomarker (Korfias et al., 2006). Primarily, S100B has been researched to determine a critical concentration level for indicating brain death following injury (Regner, Kaufman, Friedman & Chemale, 2001), and to signify the presence of post-traumatic lesions (Biberthaler et al., 2006). However, brain death and the lesions following TBI are dichotomous outcomes, and not representative of the majority of TBI prognoses. As such, there exists a need to investigate the utility of S100B in prognosticating specific recovery outcomes that are dimensional in nature, and of concern for the sequelae experienced by survivors of TBI.

The aim of the present thesis was to investigate the prognostic utility for serum S100B following TBI, with specific emphasis on neuropsychological functioning as the definable outcomes. Using a large prospective sample of TBI patients, across each classification of severity, this longitudinal population study aimed to examine the relationships that exist between S100B and current acute measures of TBI severity. Further, this thesis aimed to elucidate the ability for S100B to make prognoses for duration of post-traumatic amnesia, severity of cognitive impairment, presence of symptoms of post-concussion syndrome, and deficits in domains that are associated with quality of life.

In Study One, this thesis aimed to identify potential relationships between serum levels of S100B and the current acute measures for classifying TBI severity – namely the depth of coma, and duration of post-traumatic amnesia. Further, Study One aimed to quantify the accuracy that S100B holds in prognosing duration of post-traumatic amnesia, and whether this prognostic accuracy is superior to that of depth of coma. In Study Two, this thesis aimed to quantify the variability demonstrated in cognitive impairment at two months post TBI and the ability for S100B to prognose such impairment. Lastly, in Study Three, this thesis aimed to examine deficits in domains of quality of life and the severity of post-concussion syndrome symptomatology at six months post injury, and to investigate the ability for S100B to prognose such deficit and symptom severity.

Chapter 2

Traumatic Brain Injury: Diagnosis and Acute Management

2.1 *Definition of Traumatic Brain Injury*

Traumatic brain injury (TBI) refers to permanent or transient neurological dysfunction that has resulted from an external force to the brain. Such forces can include a blunt force trauma, or a piercing or cracking of the skull (Fortune & Wen, 1999). Further, TBI can occur without a person's head ever making direct contact with another object. Sudden acceleration or deceleration can cause the brain to violently rotate and make contact with the skull's internal architecture resulting in stretching and snapping of microscopic neural axons (Czubaj, 1996). Beyond the initial neurological tearing and shearing, a cascade of biological reactions also results from the trauma to the brain (Park, Bell & Baker, 2008). When defining TBI, an important distinction must be made between TBI and "head injury" as they are often used synonymously (De Krujik, Twijnstra, & Leffers, 2001). Although both result from contact and/or acceleration and deceleration of the head, only TBI is associated with loss of consciousness, post-traumatic amnesia, and focal neurological signs. Further, it is important to note that the definition of TBI excludes trauma that has resulted from stroke, anoxia, encephalitis and brain tumours (Koch, Merz & Lynch, 1995).

Currently, TBI is one of the most common neurological conditions, surpassed in incidence only by migraine headache and herpes zoster (Kennedy et al., 2006), and was recently referred to by the Traumatic Brain Injury Overview (2011) as the "signature wound of the Iraq and the Afghanistan wars." The number of hospitalisations for TBI is 20 times that of spinal cord injuries, and diagnoses are more prevalent than breast cancer, multiple sclerosis, and Parkinson's disease

(Flanagan, Hibbard, Riordan & Gordon, 2006). Unfortunately, despite the advances that have been made in the early detection and management of these patients' injuries, the mortality and chronic disability that can result from TBI has improved little in the last 20 years (Masson et al., 2001). Current estimates approximate that 50% of patients admitted after a "severe" TBI will be at least moderately disabled, and close to 30% will die from their injuries (Mercier et al., 2013).

Aetiological studies have demonstrated that road traffic accidents account for the largest proportion of TBIs, and that males outnumber females by three to one. This sex differential is often suggested to be due to males exhibiting higher levels of risk taking behaviour (Khan, Baguley & Cameron, 2003). Further, TBI occurs most commonly in young adults, coinciding with important life events such as completing education, career development, and starting families (Khan et al., 2003). In the younger adult population, TBI is the leading cause of brain death – and, therefore, the primary source for organ transplantation (Regner, Kaufman, Friedman, & Chemale, 2001). Beyond early adulthood, however, it should be noted that there is a second peak for TBI prevalence in older adulthood (Rapoport, McCullagh, Streiner & Feinstein, 2003). Injuries to the elderly are often due to falls (Khan et al., 2003) and they are more likely than younger adults to have substantial disabilities and fatalities in the acute period following injury, even after more mild injuries (Rapoport, Herrmann, Kiss & Feinstein, 2006).

Australian estimates suggest that 150 per 100 000 people are admitted to hospital with TBI annually (Fortune & Wen, 1999). However, Khan, Baguley and Cameron (2003) suggest that this figure most likely underestimates the true incidence of TBI because of classification and diagnostic errors, as well as the under-reporting of more mild injuries. In Tasmanian hospitals, there are over 100 emergency room

presentations of TBI each month, and the incidence of TBI in the Tasmanian population has steadily increased by approximately 6.5% each year for the last seven years (Tuck & Skilbeck, 2013). Unfortunately, the rate of TBI continues to increase despite safety improvements in the workplace, the increase of airbags in vehicles, and the enforcement of speed limits (Hergenroeder et al., 2007).

TBI is considered to be a heterogeneous disorder – classified by presentation, aetiology, and pathology. Further, it is often associated with a variety of intracranial pathologies such as intraventricular haemorrhage, diffuse axonal injury, contusion, and haematomas (Sharma & Laskowitz, 2012). As such, TBI is regularly contextualised according to the “primary” and “secondary” components of the injury. The primary insult in TBI is most often the focal damage caused by the contact injury and the resulting diffuse axonal damage (Feala et al., 2013). The mechanism and magnitude of the primary injury dictate the type and degree of the damage sustained by the patient (Sharma et al. 2012). Secondary injuries, however, occur in the minutes to hours following the primary injury. These events involve alterations and interactions in cerebral blood flow and deranged biochemical metabolism, leading to oedema, oxidative stress, inflammation, ischaemia, increased intracranial pressure, apoptosis, and necrosis (Bellander et al., 2011; Feala et al., 2013). It is recognised that these secondary injuries significantly contribute to poor outcomes following TBI, and as such, the management of TBI patients is primarily targeted towards treatment of these secondary pathologies (Stein et al., 2012). However, the heterogeneous nature of secondary injuries following TBI makes the prognosis of outcomes especially difficult (Loane & Faden, 2010). As such, it is argued that a better understanding of the cellular and molecular mechanisms implicated in

pathophysiological changes following TBI is necessary in order to facilitate more accurate prognoses for functional outcomes (Böhmer et al., 2011).

2.2 *Current Practice for Diagnosis of TBI*

Clinically, classifications of mild, moderate or severe TBI are dependent on the patient's depth of coma (often measured by Teasdale and Jennett's (1974) Glasgow Coma Scale (GCS)), duration of post-traumatic amnesia, and duration of loss of consciousness (Begaz et al., 2006; Muñoz-Cespedes et al., 2005).

Unfortunately, these measures when used to guide diagnostic evaluation are limited in their prognostic power, and are often obscured by extracranial injuries or the necessity for the patient to be sedated (Sharma & Laskowitz, 2012). Recently, predictive models have been developed to estimate likelihood of poor outcome following TBI – however, these prognostic models based on clinical/diagnostic measures have been inadequate (Walder et al., 2013).

The Glasgow Coma Scale (GCS; Teasdale & Jennett, 1976) measures patients' level of consciousness ranging from three – deep unconsciousness, to fifteen – normal alertness. A patient's score on the GCS is determined by evaluating their ability to open their eyes, and to provide verbal, and motor responses to stimulation (Hergenroeder et al., 2007). As a screening tool following TBI, the GCS is simple to complete by medical, paramedical, and nursing personnel and thus facilitates interdisciplinary diagnostic communication. Although the GCS is the current “gold standard” for diagnosing TBI severity in the acute setting, there exists an unacceptable level of outcome variability in the measure's classification populations (Ingebrigsten et al., 2000). Further, the GCS is highly susceptible to the effects of drugs and alcohol, and possesses issues with inter-rater reliability and poor

predictive power when used in isolation (Walder et al., 2013). Sharma and Laskowitz (2012) add that the validity of the GCS is compromised by intubation and sedation of the patient, and that it is difficult to ascertain the change in functioning in patients with premorbid neurological conditions. Consequently, the GCS has limited utility as a reliable tool for clinical prognoses (Beers, Berger & Adelson, 2007). In fact, Hergenroeder et al. (2008) and Korfias et al. (2007) conclude that although the GCS is currently used to stratify the magnitude and extent of brain damage, it possesses insufficient predictive value for making reliable outcome prognoses. Subsequently, a niche exists to determine adjunctive prognostic information from additional sources, given the questionable prognostic validity of the GCS and other diagnostic measures (Sharma & Laskowitz, 2012).

Raabe et al. (2003) and Mussack et al. (2006) elucidate the limitations of these diagnostic measures in the acute management setting. The authors state that TBI patients are often sedated, intubated, artificially ventilated and uncooperative with neurological examination. Further, up to between 35% and 50% of TBIs occur within the context of alcohol consumption (Brin, Borucki, & Ambrosch, 2011) and as a result, it can often be unclear in the acute setting whether neurological and neurobehavioural deficits are attributable to intoxication. Further, assessing patient history, performing reliable clinical examination (such as GCS), and obtaining a stable CT can be extremely difficult with intoxicated patients.

Beyond neurological examination, the circumstances described above often make it difficult to clinically determine the duration of patients' post-traumatic amnesia and loss of consciousness. Post-traumatic amnesia is defined as a state of clinical disorientation and inability to consolidate memory following injury (Nakase-Thompson, Sherer, Yablon, Nick, & Trzepacz, 2004). Subjectively, post-traumatic

amnesia can be ascertained by asking the patient to recall their first memory following the injury, and calculating the lapsed time. However, due to their subjective and self-reporting nature, a patient's own recollections of the duration(s) of their post-traumatic amnesia and loss of consciousness are far from being reliable sources for accurate quantitative interval- or ratio-scale information. It is important to note, however, that despite its prognostic limitations, the duration that a patient spends in post-traumatic amnesia is considered to strongly influence expectancies for immediate cognitive impairment and recovery (Lezak, Howieson, & Loring, 2004). Subsequently, objective measures of post-traumatic amnesia have been adopted into guidelines for clinical management of TBI. One such commonly adopted measure is the Westmead Post-Traumatic Amnesia scale (WPTA; Marosszecky, Ryan, Shores, Batchelor, & Marosszecky, 1998). The WPTA operationally defines duration of post-traumatic amnesia as the period of time where new memories are not consolidated, and not merely the time between the injury and the patient's first subjectively recalled memory.

At a cellular level following TBI, the blood brain barrier is known to be disrupted and permeated, thus allowing for brain specific molecules to enter the body's peripheral system. As a result, assessment of the blood brain barrier's integrity may have important implications for the patient's diagnosis and prognosis – however, the assessment of the blood brain barrier is not routine in current clinical guidelines due to the available techniques being considered too invasive. The current gold standard measurement of blood brain integrity involves the simultaneous collection of the patient's cerebrospinal fluid and peripheral serum and then analysing both samples for albumin and calculating a ratio-quotient between the samples (Blyth et al., 2009). Given the complicated and painfully invasive nature of

cerebrospinal fluid extraction from conscious patients, a less invasive indicator of blood brain barrier disruption would be beneficial to the TBI prognostic literature in order to guide clinical management for these patients.

Beyond clinical classification, for patients who have endured a moderate or severe TBI, the current emergency room standard protocol is to perform cranial computerised tomography (CT) and/or magnetic resonance imaging (MRI) if available, to investigate the presence and extent of intracranial injuries (Biberthaler et al., 2001). As a result, many hospitals over-triage to imaging departments in order to make sure that patients with intracranial complications are not missed (Calcagnile, Undén, & Undén, 2012). In fact, between 80 and 95 percent of CTs conducted after mild head injury detect no abnormalities (Ruan, Noyes, & Bazarian, 2009). Additionally, while these techniques are considered to be readily available in acute settings, the consistent use of imaging in patients with minor head injury can lead to side-effects related to radiation exposure, greater health care costs, and difficulties associated with performing the procedures in uncooperative patients (Calcagnile, Holmen, Chew, & Unden, 2013; Kondziolka, 2013). As such, it can be argued that although these techniques are patently necessary in the context of severe pathology such that the risks of not conducting imaging far outweigh the costs, the reverse may not be true when the injury is less severe.

Despite the progress in neurological monitoring technologies, CT and MRI are not efficient methods for the effective prognosis of outcome (Korfias et al., 2006; Mussack et al., 2006). Further, current imaging techniques rarely provide definitive biological indicators of secondary injury (Niogi & Mukherjee, 2010) and are statistically insensitive to mild injury (Sharma et al., 2012). Nevertheless, surgical decisions in critical care management of TBI patients remain highly dependent on

the results of neuroimaging, in conjunction with the monitoring of intracranial pressure (Yokobori, 2013). Recently, however, attention in the associated literature has turned towards reducing unnecessary imaging procedures, and investigating alternative techniques for confirmatory diagnoses.

2.3 *Acute Management of TBI*

The traditional goal for acute care of TBI in emergency departments has been physiological stabilisation of the patient, followed by minimising any further injury from secondary factors such as a drop in blood pressure leading to hypotension, a lack of oxygen leading to anoxia, or the introduction of infection in the brain through a skull fracture (Kennedy, Lumpkin & Grissom, 2006). Additionally, focus is often centred on identifying “severe” and “moderate” TBI patients who will require close monitoring or urgent neurosurgical intervention. However, approximately three quarters of patients sustain only “mild” injury, and are often discharged from the emergency room after only a brief period of observation (Begaz, Kyriacou, Segal & Bazarian, 2006). Diaz-Arrastia et al. (2013) opine that mild TBIs are difficult to diagnose as symptoms are primarily subjective, and often overlap with psychological disorders that confound the clinical picture. Further, many mild injuries go undiagnosed due to being overlooked because of more immediate medical concerns. Therefore, the practice of discharging “mild” TBI patients without admission and monitoring can be problematic, attributable to the consistent finding that despite many patients possessing otherwise identical injury factors and clinical management, their individual recovery and outcomes can be markedly different (Feala et al., 2013). In fact, patients who have sustained a mild injury still remain at risk of developing life threatening intracranial complications. As such, there exists an unmet need to

effectively manage the heterogeneity of TBI pathologies, and to personalise interventions to optimise recovery (Okonkwo et al., 2013).

Tate, McDonald and Lulham (1998) warn that vigilance is needed to detect mild injuries that can produce future neurological implications due to complications such as intracranial haematoma. The authors identify that future complications are often not being recognised in emergency departments due to the absence of expert TBI response teams and conservative screening and treatment policies. Recently, proton magnetic resonance spectroscopy, functional neuroimaging and cognitive assessments have all emerged as potential tools for investigation and identification of functional deficits following TBI (Yeo et al., 2006; Muñoz-Cespedes et al., 2005). Unfortunately, these techniques currently remain very expensive, require expert training, and are not feasible in an emergency department setting.

Theoretically, acute prognostic variables in the medical model of disease rely on a balance between sensitivity and specificity – and the preference for either a highly sensitive predictor with low specificity, or a highly specific predictor with low sensitivity is dependent on the nature of the disease. Unden and Romner (2007) state that TBI management needs a measure with very high sensitivity. In other words, the treating doctor needs a measure by which a negative result will accurately predict a more favourable outcome following their injury. Hergenroeder et al. (2007) state that although there have been improvements in response times, acute management, and survivability of TBI, there have not been any major improvements in the prognostic accuracy that can be offered to patients regarding their post-injury outcomes.

Chapter 3

Outcomes, Recoveries, and Prognoses following Traumatic Brain Injury

3.1 *Impacts and Outcomes following TBI*

TBI can cause debilitating impairments to a person's physical, cognitive, behavioural and social functioning (Hammond et al., 2004). While most people recover from these impairments without significant long-term consequences, it is well acknowledged that a substantial proportion of cases will develop persistent neurological and cognitive symptoms such as headache, impaired memory, and difficulty concentrating. The persistence of these symptoms is often referred to as post-concussion syndrome (PCS; Begaz et al., 2006). Current literature suggests that between 15 and 25 percent of individuals who sustain a TBI will suffer symptoms of PCS to the degree that their daily functioning is markedly impaired (McAllister, Flashman, McDonald & Saykin, 2006). To date, regrettably little is known about the pathophysiological aetiology of PCS (Di Battista, Rhind, & Baker, 2013). The ability to predict these outcomes would be invaluable in identifying patients who are likely to require rehabilitation, and to encourage the patient and their carers to be vigilant for reporting future symptoms (Dash, Zhao, Hergenroeder, & Moore, 2010).

The psychosocial and emotional responses to TBI can include symptoms of depression and an inability to execute effective coping strategies (Jacobson, 1995), leading to a reduction in activity levels, a decrease in social functioning and difficulty expressing needs (Rapoport et al., 2003). Economically, the occupational disability that is often associated with TBI and post-concussive syndrome can result in a considerable financial burden for the patient (Thurman, 2001; Blundon & Smits, 2000). TBI can also place economic and emotional strain on the families of the injured, particularly due to the high incidence of TBI in young adults (Flanagan,

1999). While the likelihood of a favourable recovery is grossly higher for patients who have sustained a mild injury as opposed to moderate or severe, many patients with mild TBI still report impaired ability to fulfil work and undertake family responsibilities. In fact, Diaz-Arrastia et al. (2013) argue that at a societal level, it is likely that the financial burden that results from mild injuries is at least equivalent to that resulting from severe injuries, attributable to its much higher prevalence in the population.

Some of the most disruptive symptoms experienced by patients who have sustained a TBI include behavioural issues, mood changes, impulsiveness, and increased likelihood of depression and sleep disturbance. As such, these behavioural changes can create marked complications with the patient's rehabilitation (Dash et al., 2010). Blundon and Smits (2000) refer to rehabilitative TBI patients as the "walking wounded" in that most will not suffer from permanent physical impairments that are obvious to the passing eye of the layman. However, less visible cognitive, emotional, and behavioural impairments continue to affect their daily lives. Further, each person endures a unique constellation of after-effects following a TBI and it cannot be assumed that the magnitude, effect or duration of these symptoms will be trivial or mild (Czubaj, 1996; Corrigan, Whiteneck & Mellick, 2004). Koch et al. (1995) add that these difficulties can be exacerbated for patients who do not receive appropriate diagnosis and treatment intervention. For Rapoport, McCullagh, Shammi and Feinstein (2005), and McAllister et al. (2006), the most challenging functional difficulties faced by TBI patients are impairments in their cognitive functioning.

Cognitive functioning includes a range of basic and complex domains. Basic cognitive processes include attention, orientation, and memory, whereas the more

complex processes include planning, sequencing, problem solving, and organising. Individuals use these cognitive processes in nearly every aspect of their functional lives (Blundon & Smits, 2000). TBI can affect overall cognitive processes and have a differential effect on processes within these domains (Carney et al., 1999). Inherently, there is a great need to understand the effects of TBI on cognitive functioning (Colantonio, Ratcliff, Chase & Vernich, 2004) and to obtain a prognosis for cognitive recovery as soon as possible after injury.

TBI is nearly always associated with cognitive deficits, but the extent of these deficits is considered to be highly variable (Isoniemi, Tenovu, Portin, Himanen & Kairisto, 2006). Cognitive sequelae are considered to be the most disabling of post-morbid symptoms and contribute more to persisting disability than comorbidly sustained physical injuries (Rapoport et al., 2005). Unfortunately however, Packard, Weaver and Ham (1993) suggest that cognitive symptoms are regularly overlooked by patients and their clinicians, and subjective cognitive complaints following TBI are often not objectively supported by structural neuroimaging techniques such as fMRI spectroscopy and CT scans (Koch et al., 1995; Lannoo, Colardyn, & Vandekerckhove, 1998). Consequently, discrepancies between objective findings and subjective complaints have meant that the concerns of some patients have been missed, discounted, or ignored.

The most commonly reported cognitive impairments that follow TBI occur in the domains of short-term memory, attention, executive functioning and speed of information processing; and these impairments have been well documented in the associated literature (Binder, Rohling & Larrabee, 1997; Dockree et al., 2006; Levin, High & Ewing-Cobbs, 1988; McAllister et al., 2006; Rapoport et al., 2005; Schoenhuber & Gentilini, 1989; Stuss et al., 1989). At a more finite level, specific

deficits exhibited in problem-solving, set-shifting, self-monitoring, and impulse control can have marked effects on more global cognitive domains (Sun & Feng, 2013). Further, cognitive impairments have also been found to occur in paired-associated learning and reaction time (Salmond, Chatfield, Menon, Pickard & Sahakin, 2005). However, it is the impairments to memory, information processing, and executive functioning that are considered to be most frustrating to clinical TBI populations (Chan, 2005). This finding is understandable, as all of the aforementioned domains are essential for high-functioning occupational, social and personal readaptation following TBI (Muñoz-Cespedes et al., 2005).

McAllister et al. (2006) posit that the most commonly reported memory complaints for TBI patients are often associated with working memory, which refers to the ability to hold recently acquired information in mind and to manipulate it in light of incoming material. However, the authors note that complaints are also made in reference to lower-order memory processes such as encoding and retrieval of information. This finding is supported by Freeman and Godfrey (2000), who suggest that of all of the cognitive deficits that can follow TBI, impairments to patients' memory and information processing appear the most frequently. With reference to executive functioning, Clement and Kennedy (2003) found that the domain of executive functioning is often more impaired than other cognitive processes following TBI, despite not being as overtly recognisable as deficits in memory and information processing. Further, they suggest that the recovery of this domain is comparatively prolonged. The unique recovery pattern of the executive functioning domain following TBI is often attributed to the damage caused to specific neurological structures of the prefrontal cortex (Czubaj, 1996).

Neurologically, axonal stretching, twisting, bending and snapping can be disastrous for cognitive processes (Elliot, 1996). TBIs are most often associated with damage to the frontal lobes of the brain, resulting in a variety of cognitive and neurobehavioural disturbances. The neurobehavioural disorders that often result from frontal lobe injuries include impulsivity, affect dysregulation, and impairments in self-control in accordance to social context (Czubaj, 1996). Cognitive disorders that are frequently associated with frontal lobe injury include distractibility, loss of skills surrounding abstraction and problem solving, impaired cognitive flexibility, and impaired initiation of action (Koch et al., 1995).

Damage to the prefrontal cortex is considered to be the primary cause of executive dysfunction following TBI (Rapoport et al., 2005). However, impaired executive function has also been found with trauma to subcortical structures such as the thalamus, limbic system, basal ganglia, and cerebellum (McAllister et al., 2006). As a result, executive dysfunction is highly prominent in the cognitive sequelae of TBI due to the often diffuse nature of traumatic injury.

Elliot (2003) posits that successful executive processing involves subcortical and posterior cortical regions working in concert with the prefrontal cortex. With reference to working memory, this domain is likely to be impeded if damage has been sustained by the temporal lobes and the pre-frontal cortex (Soeda et al., 2005). Injury to the left temporal lobe will often result in impairments to verbal learning and memory, auditory attention and discrimination, and disturbances in language – whereas damage suffered by the right temporal lobe will often result in impairments in visio-spatial perception (Czubaj, 1996). Unfortunately, the outcome measures that are traditionally utilised in prognostic research are insufficiently sensitive to the unique neuropsychological impairments that most commonly result from TBI. As

such, any translational implications that are made from such studies are limited in ascertaining true predictors of neuropsychological recovery following TBI.

3.2 *Neuropsychological Recovery following TBI*

For the majority of TBI patients, good recovery outcomes are expected for the first months following injury. Unfortunately, however, not enough is known about the nature of recovery and residual impairment across the stages of recovery (McAllister et al., 2006). Further, Millis et al (2001) suggest that there is more to be understood regarding the extent to which severity and premorbid factors influence impaired cognitive recovery following TBI. Millis and colleagues (2001) argue that prior studies have failed to address these research questions due to small samples, insufficient power, inadequate follow-up criteria, or poor statistical design. Salmond et al. (2005) add that many of these studies have relied on insensitive measures of cognitive deficit. Hence, there is a need for research to utilise measures with robust reliability and predictive validity (Beers et al., 2007), and these measures should be applied following discharge, across the course of recovery (Van Baalen et al., 2006). Unfortunately, robust cognitive measures are often considered to be “elegant” measures of outcome, and too expensive or difficult to incorporate into TBI research design (Lo, Jones, & Minns, 2009). Further, by requiring in-person follow-up evaluations in longitudinal TBI research, the attrition rate is often markedly impacted (Topolovec-Vranic et al., 2011).

Beyond the acute-phase of rapid recovery of consciousness, orientation, and continuous memory, not enough is known for improvements made over time (Hammond, Hart, Bushnick, Corrigan, & Sasser, 2004). Further, there is a clear variability in outcomes and recovery patterns across individuals with equivalent

injury profiles. Although it is generally accepted that the long-term prognosis for mild and moderate cognitive recovery following TBI is good, it is also the case that there exists a proportion of these patients who will experience limited and incomplete recoveries (McAllister et al., 2006). Studies have even found that some patients may actually demonstrate a steady decline, even at a younger age – however, these trajectories are exceptionally rare (Teasdale, Murray & Nicoll, 2005). Given the range of potential outcomes, the accurate determination of which patients will spontaneously recover and which will endure long-lasting impairments is notoriously difficult (Van Baalen et al., 2006).

Muñoz-Cespedes et al. (2005) speculate that there are two distinct mechanisms that influence cognitive recovery following TBI. The first is a neural mechanism that operates from the instant that the injury occurs. The authors suggest that this mechanism will recover or reach a plateau within six months following the injury. Secondly, the authors propose a personal-agency mechanism, implying that improvement beyond six months is dependent on the patient's development of compensatory strategies and adaptation to the residual effects of their injury.

It is often suggested that recovery of premorbid cognitive functioning is expected to occur for most TBI patients (Elliot, 2003). This is supported by the results of Yeo et al. (2006) who found that the majority of associated neurometabolite recovery takes place in the first two months following the injury, with additional slow minimal improvements up to six months. Muñoz-Cespedes et al. (2005) add that the recovery of premorbid social behaviour and personality traits also occurs predominantly within the first six months following a TBI.

Beyond the six-month period, Hopkins and Jackson (2006) found that minimal improvements are made in neurocognitive functioning between six-months

and twelve-months, with some patients improving for up to three-years before their trajectory enters a plateau. Further, research conducted by Millis et al. (2001) found that some patients exhibited neurocognitive impairments even at five years post-injury, leading the researchers to speculate that neurocognitive sequelae may be permanent for some patients. Patients who complain of long-term neuropsychological and somatic symptoms are regarded as suffering from persistent PCS. The associated literature reports that the risk factors for PCS include duration of post-traumatic amnesia and loss of consciousness (Iverson, Lovell, & Smith, 2000), age, sex, a previous incidence of TBI (Binder, 1997), and comorbid depression (Busch & Alpern, 1998). However, none of these diagnostic and premorbid factors have been proven to be reliable as a true prognostic variable for the development of PCS (Stapert, et al., 2005).

3.3 *The Need for Accurate Prognostics*

Beers et al. (2007) have stated that increasing the accuracy of prediction of neuropsychological outcomes following TBI is essential. The authors argue that prognoses based on more reliable prediction methods can provide patients and their carers with accurate information and realistic expectations for outcome, and facilitate effective and appropriate referrals to rehabilitation services. Further, de Krujik et al. (2002) suggest that time and expenses that are invested in follow-up sessions with specialists would be drastically reduced if there was a reliable means to identify those who are most expected to exhibit persistent symptoms while they are still in the context of an acute presentation. If appropriate rehabilitation services are made available to these patients, it may be possible to reduce the number of patients whose conditions become chronic, and to minimise the financial costs that they encounter

during rehabilitation (Savola & Hillborn, 2003). Townend, Guy, Pani, Martin and Yates (2002) and Hammond et al. (2004) add that effective clinical prognoses can determine practical resource allocation, and therefore, maximise the resources and treatment options that are made available to the patient.

Identification of a highly sensitive prognostic variable for TBI could also be of use in the medico-legal context. Ingebrigsten et al. (1999) suggest that such a variable may prove that neuropsychological disability or impairment after a traumatic event is in fact due to the TBI, and not to stress disorder, systemic injury or other causes. Hergenroeder et al. (2008) add that in the example of shaken baby syndrome, unreported trauma in concert with non-specific indistinguishable symptoms (irritability and vomiting) may result in the abuse going undetected. Such a prognostic tool would indicate the need for brain imaging and the implementation of abuse reporting procedures. Unfortunately, the diagnosis, prognosis, and treatment of TBI are hindered by the current lack of such a variable. Contemporary methods of imaging (CT, MRI, and fMRI) rarely provide definitive indicators of biological damage following TBI (Okonkwo et al., 2013).

Due to TBI causing diffuse axonal and small vessel injury that is often undetectable by computerised tomography, emerging research has begun to investigate the relationship between brain-related proteins and TBI outcome (Vos & Verbeek, 2002; Begaz, Kyriacou, Segal, & Bazarian 2006). More specifically, the current research has focussed on the biochemical changes that result from structural damage to neuronal cells. This damage causes a leakage of certain proteins into the patient's extracellular matrix and cerebrospinal fluid (Zemlan et al., 2002). As a result, if the blood-brain barrier is damaged, these proteins are released into the

patient's peripheral circulation, where they can be sampled and measured (Begaz, et al., 2006).

Clearly, the appropriate and accurate identification of patients that are likely to experience post-traumatic complications is of high clinical importance and, as a result, prognostics is the direction of current neuropsychological TBI literature. The ability to focus early intervention resources on these high-risk patients can only be achieved by a reliable prognostic indicator of sustained damage. The current trend for prognostic medical research has veered away from measures that require subjective responses and recollections from the patient (such as GCS, and duration of post-traumatic amnesia and loss of consciousness) towards investigating the differential benefit offered by objective pathophysiological measures for outcome (Berger, Beers, Richichi, Wiesman, & Adelson, 2007). However, as de Boussard et al. (2005) suggest, the pathophysiological basis for cognitive impairment is far from clear in neuropsychological literature. As a result, neuropsychological outcome research is beginning to incorporate biomedical variables as constituents of prognostic study.

The biomedical sequelae that follows TBI is an increasingly active area of neuropsychological research, and may provide a new assessment of severity with robust prognostic validity that is immediately available at the time of injury (Begaz et al., 2006). Consequently, a comprehensive understanding of the post-traumatic release patterns of brain-specific proteins could help to identify patients who are at risk of developing long-term neuropsychological dysfunction (Herrmann et al., 2001). The identification of a brain-specific protein with prognostic value would constitute what is referred to in medical science models of disease as a "biomarker." Currently, no serum biomarkers are routinely used in clinical presentations of TBI

(Okonkwo et al., 2013). Nevertheless, because of the medical need outlined above, there has been a heightened scientific interest in identifying molecular biomarkers of TBI.

Chapter 4

Biomarkers and Traumatic Brain Injury

4.1 Biomarkers

In medical sciences, biomarkers are used to provide diagnostic or prognostic information towards an altered physiological condition or disease state (Gonçalves, Leite & Nardin, 2008). Further, biomarkers provide objective indications for effective patient management and can detect disease and injury that may have been overlooked in routine clinical evaluation (Unden & Romner, 2009). As such, Kondziolka (2013) states that nothing transcends medical disciplines more than the concept of defining biomarkers – adding that a desire resides in all physicians to be able to quantify a measure that can accurately predict the presence or absence of an active pathology and thus be used to forecast prognoses for recovery.

Biomarkers can be detected and monitored in a variety of bodily fluids and tissues, and they may be detected in the human system by specifically contrasted imaging techniques, or alternatively, through laboratory analysis of the specific fluid or tissue (Jeter et al., 2013). The majority of established biomarkers are evaluated in the serum component of a patient's peripheral blood sample. Blood serum is the component of a collected blood sample that is neither a clotting factor nor a blood cell – rather, it is the blood plasma with the fibrinogens removed. The use of blood as

the source for biomarkers is attractive due to its accessibility, low invasiveness, minimal cost, and simple collection and processing (Hergenroeder et al., 2008).

Biomarkers are widely used in many areas of medicine as a means of monitoring progression of pathology and response to treatment. Subsequently, biomarkers have gradually entered the domain of clinical neurotraumatology (Thelin, Johannesson, Nelson, & Bellander, 2013). Potentially, an effective biomarker of TBI would identify the presence of processes that are difficult to image, such as diffuse axonal injury, and provide evidence of any ongoing tissue damage (Sharma & Laskowitz, 2012). Subsequently, the effective utility of biomarkers in TBI could help streamline patient priority, reduce waiting times, and determine which patients require emergency CT imaging (Egea-Guerrero, Murillo-Cabezas, & León-Carrión, 2013). Ohrt-Nissen (2011) add that incorporating biomarkers into the management of TBI could also reduce the number of unnecessary CT imaging, and inherently lower the rate of negative findings in CTs conducted on TBI patients. In the context of continuing life support, Vos et al (2010) caution that when the withdrawal of treatment is being considered, physicians must be confident that a biomarker result on which the decision is being based is highly specific for the particular outcome (i.e., death or persistent vegetative state) and that the number of false-positives is extremely low.

In current medical practice, serum biomarkers can be used to provide clinicians with information regarding the severity and course of the pathology of disease (Korfias et al., 2007). At present, serum biomarkers are common adjuncts to the clinical management of many conditions whereby the detected levels of specific biomarkers have been shown to correlate with the clinical severity of organ damage and functioning for a variety of pathologies (Shore et al., 2007). Some examples of

established biomarkers include creatinine for renal failure, troponin I for myocardial injury, prostate specific antigen for prostate cancer, and amylase for pancreatitis. These biomarkers have been shown to be specific to the cells of their respective organ systems and are subsequently used to ascertain information about the diagnosis, prognosis, and effect of treatment for the patient (Raabe et al., 2003). In fact, Goyal et al. (2013) state that proteomic biomarkers are currently part of mainstream clinical care for almost every organ system except the brain. Ideally, for the biomarker to be attractive for clinical use it should be easy to access, fast and inexpensive to process, and simple to interpret (Oknonkwo, 2013).

Sharma and Laskowitz (2012) argue that candidate biomarkers (for any condition or disease) should be subjected to rigorous study in order to elucidate and define their ability to provide adjunctive information in the clinical presentation for which they will be used. As such, the model for biomarker development in TBI can be framed analogously to models for any other medical presentation. In the oncological prognostic literature, five phases of biomarker development have been identified. Pepe, Etzioni and Feng (2001) state these phases as: 1. Identifying promising molecules; 2. Clinical assay development and validation; 3. Retrospective longitudinal studies on patients with known outcomes; 4. Prospective screening studies to determine whether a condition can be identified; and 5. Measurement of the impact of screening on reducing the burden of the condition on the population. Logically, these phases could be applied to the study of TBI. However, Hergenroeder, Redell, Moore and Dash (2008) state that while biomarker profiling has proven to be useful in detecting oncological pathologies, the utility for biomarkers in the management of TBI is at an early stage of investigation. Papa et al. (2013) add that despite the infancy of the application of proteomic biomarkers in

TBI, the ongoing advances in the understanding of human neurobiochemistry can provide insight into the pathways by which TBI can be understood and the context by which biomarkers can be applied.

Hergenroeder et al. (2008) posit that the identification of a specific TBI biomarker would aid in the diagnoses of TBI and polytrauma, identify patients at risk of developing secondary complications, and potentially, make accurate predictions for patients' neuropsychological recoveries. Sun and Feng (2013) add that, with advancing technologies, biomarkers may elucidate the mechanisms that are involved in TBI and identify new strategies for therapeutic intervention. However, the translation of TBI biomarker research into clinically useful prognostic tools has not been successful to date (Lo et al., 2009). As such, several challenges still exist in effectively modelling the heterogeneity that is observed in TBI outcomes (Niyonkuru et al., 2013). Attempts to resolve these challenges are currently being facilitated by an increased focus on identifying biomarkers for outcome prognoses, with a growing emphasis on translational application of the findings. One such application would be elucidating the role of biomarkers in the prognosis of neuropsychological impairment following TBI, by exploring the mechanisms that underlie the changes in the respective biomarker of injury (Sun & Feng, 2013).

Indeed, without exception, biomarker research has been the largest growing area in the domain of TBI prognostic literature. Figure 4.1 below depicts the increase in peer-reviewed publications per year containing "biomarker" and "brain injury" in key words for the last twenty years (source: <http://www.pubmed.com>).

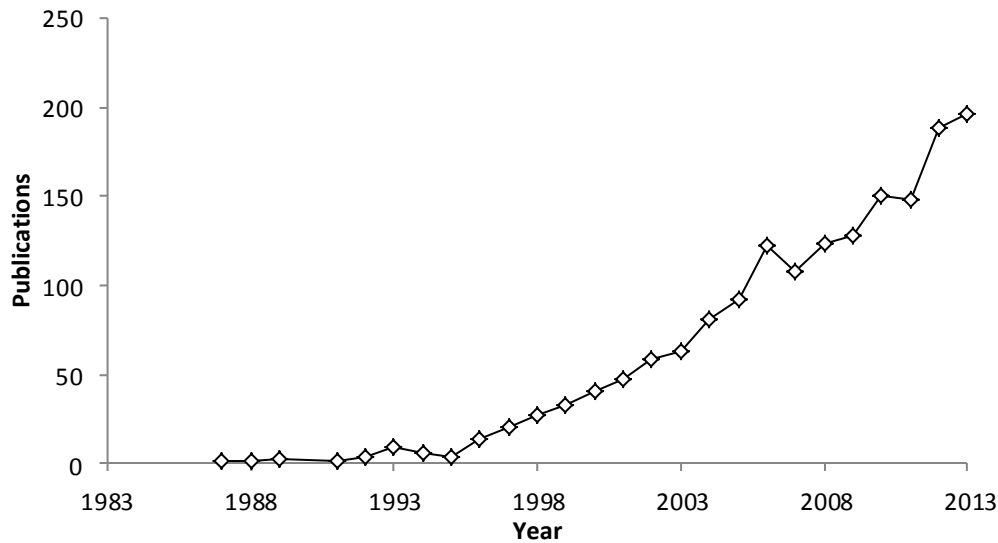


Figure 4.1 “Brain Injury” and “Biomarker” articles published annually

Shore et al. (2007) argue that the increase in recent biomarker research for TBI is attributable to the fact that no single definitive biomarker currently exists for indicating brain damage severity. The prospect of establishing a routine neurobiochemical marker for TBI has led to a growth of experimental and clinical neurotraumatology studies being conducted by biochemical scientists, neurologists, and haematologists – all incorporating hypothesised biomarkers of brain damage into their research design (Herrman et al., 2001). Subsequently, the inclusion of an ideal biomarker into the evidence-based guidelines for TBI diagnosis and prognosis is eagerly anticipated across many medical sciences (Petzold et al., 2002).

Unfortunately, however, Papa et al. (2013) suggest that the flurry of research in the area is limited by small sample sizes, unstandardised sample collection practices, and disparate outcome measures. Inherently, more work is necessary to clarify the unique diagnostic and prognostic utility of candidate proteins in order to classify them as potential biomarkers for TBI (Nyonkuru et al., 2013).

In broad terms, the Biomarkers Definitions Working Group (2001) state that a biomarker should indicate the presence or absence of disease/injury, and should be able to stage or classify its severity. More topically, Pelinka, Toegal, Mauritz and Redl (2003) state that an ideal biomarker for TBI should be highly specific for the brain, highly sensitive for TBI, appear rapidly in the serum, and be released in a time-locked sequence with trauma. Yokobori et al. (2013) expand this by stating that the specificity of an ideal TBI biomarker would indicate that TBI is uniquely present and accurately reflect the severity of the damage, and the sensitivity of the biomarker would be sufficient to indicate that changes in the marker are easily identifiable. Korfias et al. (2006) add that an ideal biomarker should also have no age or sex variability, and a consistent relationship between the serum concentration and the level of specific tissue damage. The criteria and standards for an ideal biomarker of TBI are expanded by Weber and Maas (2007). The authors argue that the biomarker should originate in the central nervous system with no contribution from extracerebral sources, release from damaged neurons or glial cells, and express an unlimited passage through the blood-brain barrier.

Understanding the role of the blood brain barrier and its functional status in secondary pathologies is crucial to establishing biomarkers for TBI. Unfortunately, current imaging techniques lack the resolution that is necessary to accurately determine the functional status and integrity of the blood brain barrier's anatomical structure. The blood brain barrier consists of only a single layer of capillary endothelium that, when intact, prevents the diffusion of most water-soluble molecules over 500 Daltons (Da). When damaged however, brain related proteins are able to enter the peripheral circulation (Blyth et al., 2009). Conversely, damage also permits entry of blood-borne materials into the brain. The intrusion of these materials

into the brain has been linked with increased intracranial pressure, and altered biochemical homeostasis following TBI (Dash et al., 2010). It is the detection of brain specific proteins in the peripheral system, rather than the opposite, that has attracted the focus of prognostic research. Logically, the presence and magnitude of any brain related protein in the peripheral system would indicate permeation of the blood brain barrier and thus, measuring the protein may facilitate diagnosis in an otherwise equivocal presentation.

Recently, several biochemical substances have been studied to find such an ideal marker for indicating brain cell damage, however, the associated research has commonly shown that these markers both lack specificity and fail to provide reliable information for the diagnosis and treatment of TBI (Rainey, Lesko, Sacho, Lecky & Childs, 2009). This view is supported by Ucar, Baykal, Akyuz, Dosemeci and Toptas (2004) and Raabe et al. (2003) who add that lactic dehydrogenase, creatine kinase-BB isoenzyme, neuron-specific enolase, and myelin basic protein have yet to prove their utility as biomarkers of TBI. However, Hergenroeder et al. (2008) suggest that these biomarkers can provide indications for secondary pathologies that are often associated with TBI, such as intracranial pressure.

At present, there is insufficient clinical research to support biomarkers being used in a diagnostic framework to distinguish focal and diffuse injury (Yokobori et al., 2013). Calcagnile et al. (2013) add that identification of any brain specific biomarker for acute and chronic secondary pathologies could result in earlier diagnoses and improved accuracy of prognoses. Further, potential exists for biomarkers being used to select between targeted neurochemical interventions in order to treat damage to neurocellular networks (Papa et al., 2013). For this reason, prognostic biomarkers can potentially have a twofold purpose in the context of TBI –

namely, to predict recovery, and to stratify the risk for specific secondary pathologies (Di Battista, Rhind, & Baker, 2013).

The current direction of TBI biomarker research has focussed towards the investigation of the S100 calcium binding protein B (S100B) (Ucar et al., 2004), as it has been shown to fulfil the criteria of an ideal brain damage biomarker (Korfias et al., 2006). In fact, Hergenroeder et al. (2008) state that S100B is now the most studied TBI biomarker to date and research into this protein is many years ahead of the work with other biomarkers. Primarily, S100B has been researched to determine a critical concentration level for indicating brain death following injury (Regner, Kaufman, Friedman & Chemale, 2001), and to signify the presence of post-traumatic lesions (Biberthaler et al., 2006). Under these clinical applications, S100B has been shown to be a sensitive and easily monitored biomarker for determining the pathophysiology of patients' brain injuries (Savola et al., 2004). However, brain death and the lesions following TBI are dichotomous outcomes, and not representative of the majority of TBI prognoses. As such, there exists a need to investigate the utility of S100B in prognosticating specific recovery outcomes that are dimensional in nature, and of concern for the sequelae experienced by survivors of TBI. More specifically, Sharma and Laskowitz (2012) opine that a truly effective biomarker of TBI would provide clinical information regarding cerebral responses to acute interventions, and identify patients at risk of developing long-term neuropsychological impairment following their injury.

4.2 *S100 Calcium Binding Protein B (S100B)*

The S100 proteins were first identified by Moore (1965). The name of the protein arrangement is derived from its solubility in ammonium sulphate, which is at 100% at a neutral pH (Fillipidis, Papadopoulos, Kapasalaki, & Fountas, 2010). The protein is composed of two subtypes, alpha and beta (or A and B). The alpha subtype is found in striated muscles such as the heart and kidneys, whereas the B subtype is found in high concentrations in astroglia. S100B is a calcium-binding protein that is produced by Schwann and glial cells (Biberthaler et al., 2001; Kleindeist & Bullock, 2006) – however, it can also be found in small traces in several non-neuronal cells such as adipocytes (fat cells), chondrocytes (cartilage cells), skin, and glioblastoma and melanoma cells (Yokobori et al., 2013). Despite its expression in these extracranial sources, research conducted by Pham et al. (2010) clearly illustrated that changes in serum levels of S100B post TBI are dictated solely by extravasation across the damaged blood brain barrier.

S100B has been shown to be important to the regulation of neuronal cellular homeostasis by exerting autocrine and paracrine functions to astrocytes and neurons in the brain. Astrocytes and glial cells are prevalent in the white matter and the basal ganglia of the brain, and the release of S100B from these sites following injury appears to reflect immediate cellular damage in the brain's white matter (Sedaghat & Notopoulos, 2008). More specifically, the S100B protein has been shown to be related to the structures of the corpus callosum, the anterior forceps, and the superior longitudinal fasciculus. (Streitbürger et al., 2012). The S100B protein has a homodimeric structure, where each beta monomer is approximately 10.5 kDa in weight (Gonçalves et al., 2008), this structure regulates protein phosphorylation and is said to be fundamental to the proliferation and neurotrophism of astroglial cells

(Sedaghat & Notopoulos, 2008). As such, at a cellular level, S100B has been implicated in the modulation of learning and memory due to the link between the proliferation of astroglial cells and cognitive performance (Ellis, Willoughby, Sparks & Chen, 2007).

Following brain injury, S100B is hypothesised to be released from the brain into the bloodstream, via cerebrospinal fluid, due to permeation in the blood-brain barrier caused by the injury (Beers, Berger & Adelson, 2007). Ordinarily, S100B does not cross the intact blood-brain barrier (Hergenroeder et al., 2008), attributable to its molecular weight described above. Subsequently, the serum level of the S100B protein detected following TBI is indicative of a systemic inflammatory reaction, causing a decrease in the integrity of the blood-brain barrier, and more severe neurological disruption (Hergenroeder et al., 2008; Sedaghat & Notopoulos, 2008). Logically, the theory would follow that a patient's level of serum S100B after injury should be inversely correlated with their outcome.

At a microscopic level, the release of S100B to the peripheral blood stream following TBI is suggested to be the result of two processes. The first process suggests that astrocytic structural and membrane integrity is compromised as a result of injury related stretching. This process has been reported by electro-microscopic studies of stretch-injured astrocyte cultures. Inherently, increased S100B levels reflect the degree to which TBI related glial damage causes astrocytic reactions, also referred to as reactive astrogliosis (Bohmer et al., 2013). The second theorised process posits that the adenosine triphosphate nucleotides and glutamate amino acids are also released after TBI. Subsequently, the release of S100B may be partly attributable to astrocytic receptor activation by adenosine triphosphate and glutamate (Weber & Maas, 2007). As such, Michetti et al (2012) state that S100B levels are not

merely a consequence of cell damage, but also an indicator of secondary processes. It is therefore the secretion of the protein and its participation in secondary events that could offer pathogenic and therapeutic indications for TBI management and prognoses.

The typical release pattern of S100B following TBI or ischaemic brain conditions involves an early peak immediately after the primary brain trauma, due to the increased permeability of the blood-brain barrier (Biberthaler et al., 2001; Raabe et al., 2003). Immediately following injury, the level of S100B in serum may rise up to concentrations of between 5-20µg/L. This initial high reading is attributed to damaged brain cells and an opening of the blood-brain barrier that only occurs in the first minutes after trauma (Raabe et al., 2003). The peak level of S100B in the peripheral system is said to remain for one to three hours post injury, before declining to an undetectable level (Begaz, Kyriacou, Segal & Bazarian, 2006). Townend et al. (2006) attribute this fall as either the closing of the blood-brain barrier, or the cessation of S100B production at a cellular level. Ingebrigsten, Waterloo, Jacobsen, Langbakk and Romner (1999) found that 45% of their sample population had declined to undetectable levels within six hours of their injury. However, Raabe et al. (2003) found that S100B can remain elevated for days if the patient is suffering from progressive secondary brain damage or chronic barrier disruption.

As a result of the established release pattern for S100B following TBI, Townend et al. (2006) suggest that the timing for sampling patients' blood is critical, and should be performed as early as possible following injury. Begaz et al. (2006) add that unless sampling is performed within three hours of injury, the potential utility for S100B in the emergency setting is critically compromised. Similarly,

Raabe et al. (2003) suggest that it is critical to know the time of the injury for accurate interpretation and outcome prediction. Serial-sampling research conducted by Woertgen, Rothoerl and Brawanski (2002) concluded that presentation levels of S100B, that are less than one hour post-injury, are the most reliable in correlating with injury severity, and suggest that future outcome researchers adopts this time-point as the predictor variable.

Wiesmann, Missler, Gottman and Gehring (1998) were the first researchers to publish findings to suggest that serum concentrations of S100B are not influenced by a patient's blood-alcohol level. Vos and Verbeek (2002) attribute this finding to their conclusion that the astroglial compartment of the brain is not affected by alcohol intoxication, and resultantly, serum S100B concentrations are unaffected by alcohol intake. This finding has been supported by the research of Korfiat et al. (2007) and Unden and Romner (2009). More recently, Calcagnile et al. (2013) conducted a prospective study of alcohol and S100B levels in 621 TBI patients. The researchers concluded that the presence and magnitude of alcohol had no effect on S100B levels, irrespective of whether the consumption was derived from patient history or from objective blood ethanol levels. The authors concluded that S100B can be used reliably in TBI regardless of intoxication. Similar conclusions were made in a prospective study on 107 TBI patients, using CT imaging as the dependent variable (Wolf et al., 2013), and also in a study that stratified 160 patients across four levels of intoxication (Lange, Brubacher, Iverson, Procycyshyn, & Mitrovic, 2010). Given that alcohol use is a significant risk factor for TBI (Lange et al., 2010) and approximately 35% to 50% of patients with TBI are intoxicated to some degree (Brin et al., 2011), all of the findings mentioned are especially pertinent to TBI biomarker research. Taken together, the immunity that S100B exhibits towards alcohol provides

additional clinical utility in the context of TBI beyond the demands of regular biomarker criteria.

Further advantages of S100B as a biomarker include that it can be measured in the arterial and venous blood systems, it is not affected by haemolysis, and after collection it remains stable for several hours at room temperature without the need for immediate analysis or refrigeration (Korfias et al., 2007). Additionally, Unden and Romner (2009) suggest there may be significant cost-benefit potential should S100B be proven to be a true biomarker of TBI pathophysiology. Currently, the total cost of performing an S100B assay is 10% of the cost of performing cranial CT imaging and approximately 2-5% of the cost of one day of inpatient observation. Hergenroeder et al. (2008) add that cost savings can also be made by identifying appropriate management procedures for patients such as monitoring intracranial pressure, and referring for specific neurobehavioural and neuropsychological interventions.

Gonçalves et al. (2008) note that several biochemical features of S100B are not well categorised, and subsequently, controversies exist regarding its clinical function and utility. Weber and Maas (2007) add that despite the growing body of evidence demonstrating an association between S100B and poor outcomes following TBI, researchers should be aware that proof of association is not necessarily a proof of causation. The implications of findings and models of causation are the current grounds for debate regarding serum S100B levels following TBI.

Most commonly, debate exists as to whether S100B is neuroprotective or neurotrophic in its actions following TBI (De Boussard et al., 2005). Research conducted by Pleines et al. (2001) suggests that S100B stimulates neuronal survival and glial cell production – however, its upregulation of calcium binding potentially

contributes to post-traumatic neurotoxicity. The researchers concluded that their study could not determine whether S100B is detrimental or beneficial for the injured brain, or whether it simply reflects the severity of injury. Similarly, Kleindienst and Bullock (2006) found that while S100B had a neurotrophic effect on paracrine and neurite outgrowth in rats, administering S100B to post-injury rats actually improved the rats' overall cognitive performance. Gonçalves et al. (2008) suggest that the dualistic view of S100B as being either “good” or “bad” simplifies clinical practice and delays the comprehension of the role that S100B plays in physiological conditions and brain disorders.

4.3 *S100B and TBI*

As with most biomedical science, the literature associated with S100B and TBI has its foundations in animal models of injury. In an exploratory rodent experiment, rats with S100B surgically infused into their hippocampi demonstrated dose-dependent increased performance in a long-term memory task, compared with sham control following TBI (Mello e Souza, Rohden, Meinhardt, Gonçalves, & Quillfeldt, 2000). Conversely, however, Winocur, Roder, and Lobaugh (2001) found that transgenic mice with overexpressive S100B demonstrated learning and memory impairment, compared to nontransgenic controls. As such, it is clear that animal experiments do not always reproduce the same results across studies. Feala et al. (2013) argue that these discrepancies are attributable to variations in species, injury type, and time course of sample collection. Further, the emphasis on sample size and statistical power is often not the same for “bench top” as opposed to “bedside” research (Marincola, 2003). Lastly, with specific reference to TBI, Walder et al. (2013) state that by contrast to animal experiments, patients with TBI have a

heterogeneous pattern of injury, and the different injuries sustained that result in different biomarkers being released make it difficult for translational associations to be made with clinical outcomes in human models.

From a purely diagnostic perspective, S100B has been shown to be correlated with the existing classification measures of TBI. Böhmer et al. (2011) demonstrated that S100B levels are significantly higher in patients who have experienced a severe TBI, compared to healthy controls. Beyond “severe” classification, Savola et al. (2004) found that S100B was hierarchically correlated across mild, moderate, and severe classifications of TBI. The researchers added that their results indicated a high negative predictive power suggesting that a normal S100B level, recorded shortly after injury, excludes significant brain injury pathology with high accuracy. Similarly, S100B has been shown to differentiate concussion from superficial scalp injury with 94% sensitivity and 100% specificity (Matek, Vajtr, Krška, Springer, & Zima, 2012). Further, Vajtr et al (2012) demonstrated that S100B levels can be used to differentiate diffuse axonal injuries from focal injuries.

Beyond routine classification measures, a study conducted by Korfiatis et al. (2007) found that high S100B levels were also correlated with other diagnostic measures such as severity of papillary status and CT examinations. In fact, a number of studies have investigated the relationship between S100B and diagnostic CT examinations, and this domain is arguably the most prolific area of translational S100B research. The rationale for these imaging studies is that biomarkers could be used to rule out TBI, allow better use of triage resources, save on operational costs, and avoid exposing patients to unnecessary radiation from imaging (Mercier et al., 2013).

Using the Marshal CT classification system (Marshal et al., 1992), Herrmann et al. (2001) found that S100B levels were significantly correlated with CT severity in a cohort of TBI patients. Further, by comparing CT scans of 60 TBI patients in which two thirds had “positive” CTs (i.e., a remarkable pathology), Cervellin et al. (2012) found that S100B levels were significantly higher in the CT positive group. The researchers demonstrated that S100B levels indicated positive CT results with 100% sensitivity and 58% specificity. Similarly, using only mild TBI patients, studies conducted by Morochovič et al. (2009) and Bazarian et al. (2013) which respectively found that S100B levels indicated abnormal (positive) CT scans with 90% and 83% sensitivity, and 34% and 30% specificity.

As mentioned earlier in the discussion of TBI diagnosis, the current gold standard measurement of blood brain integrity involves calculating a ratio-quotient between albumin levels found in cerebrospinal fluid and peripheral serum. A study by Blyth et al. (2009) found that serum S100B levels held a significant relationship with albumin quotient levels. Further, the study showed that S100B could classify abnormal albumin quotient values with 80% sensitivity and 90% specificity. The researchers concluded that serum S100B elevations were therefore not only indicative of physical blood brain barrier disruption, but also of deranged osmotic functioning of the barrier following TBI.

S100B has also been found to be correlated with secondary pathologies such as increased intracranial pressure, cerebral hypoxia, and intracranial haemorrhage. Research conducted by Petzold et al. (2002) illustrated that S100B is a sensitive biomarker for the development of elevated intracranial pressure, and suggest that it could be used as an indicator for follow up CT for patients who may have been discharged as a result of “mild” classification, in order to mitigate risk of increased

intracranial pressure. These results were replicated by studies conducted by Nylen et al. (2008) and Muller et al. (2007). Muller et al.'s discussion cautioned, however, that S100B should not entirely replace clinical examination or CT for patients with head injury who have suspected increased intracranial pressure. Beyond intracranial pressure, Stein et al. (2012) found that serum S100B levels were a highly specific, yet insensitive, indicator of cerebral hypoxia. The researchers conclude that despite only modest associations, the finding that S100B is detectable prior to the onset of cerebral hypoxia is important for the prediction of impending pathological events. Further, Wolf et al. (2013) demonstrated that S100B was able to identify future intracranial haemorrhage – however, the authors do not support the use of biomarkers in isolation to make such a diagnosis.

As demonstrated by the diagnostic literature reviewed above, S100B possesses clinical utility in diagnosing and differentiating TBI, identifying secondary pathologies, and stratifying the severity of the patient's injury. It is important to note, however, that the severity classification of an individual's TBI does not necessarily equate to the severity of their future impairment – in short, diagnosis does not equal prognosis.

For all TBI patients admitted to hospital, an unfavourable prognosis such as death, persistent vegetative state, or chronic severe disability could be the case for over 20% of these presentations (Egea-Guerrero, Murillo-Cabezas, & León-Carrión, 2013). Further, TBIs are among the most common cause of violent deaths, and are commonplace following violent assaults (Ondrushka et al., 2013). As such the utility of S100B in medico-legal and forensic settings is an emerging research area in the associated prognostic literature. One such application is the potential role that S100B could facilitate in objectively determining the presence of TBI, either accidental or

abusive, in children and babies. Due to these patients presenting with non-specific symptoms, and often, the inability to communicate the aetiology of their injury, a minimally invasive serum biomarker could be used to rule out TBI pathology (Papa et al., 2013).

A second medico-legal application for S100B as a biomarker for TBI relates to its use in prognosing brain death, and thus, facilitating decision-making related to organ transplants. Unfortunately, the number of organ donors in developed countries has progressively declined despite recent improvements in the process of organ donation and transplants. In order to identify potential organ donors, transplant teams require strict, efficient indicators of irreversible vegetative coma. Regression analyses conducted by Dimopoulou et al. (2003) found serum S100B to be an independent and highly predictive biomarker for trauma-induced brain death. Further, odds ratio analyses indicated that for every 1 µg/L of S100B detected on admission, the probability for deteriorating to brain death more than doubles. Similarly, Raabe et al. (2003) found that serum S100B values that are greater than 2 µg/L present a high likelihood for a brain death diagnosis. More recently, Egea-Guerrero et al. (2013) suggest that S100B could be used to detect patients at risk of brain death, and to identify them as potential donors. The authors studied 140 survivors of severe TBI (based on GCS) and found that the 16 patients who developed brain death had significantly higher S100B concentrations, and that by using a cut-off of .37 µg/L, brain death was prognosed with 85.7% sensitivity and 73.9% specificity.

Egea-Guerrero et al.'s (2013) finding is supported by the earlier results of Böhmer et al. (2011) who found that the CSF levels of S100B taken between four and seven days post injury in people who died from TBI ($n=5$) were significantly

higher than those who survived their injuries ($n=15$). These findings have some inherent implications for the management of life-support for TBI patients. As mentioned previously, many TBI patients are young and often have no prior morbidity – as such the decision to withdraw life sustaining treatment needs to be based on sound prognostic modelling of survival likelihood (Mercier et al., 2013).

With reference to post-mortem confirmation of TBI, Ondrushka et al. (2013) found that S100B is useful in characterising cause of death and survival time at forensic autopsy. The researchers found that both serum and CSF levels of S100B were significantly elevated in autopsy cases where TBI was known to be the explicit cause of death. Further, it was reported that serum levels of S100B were significantly inversely correlated with survival time following fatal head injuries. The researchers concluded that common autopsy practice could potentially incorporate forensic biochemistry in the event of controversial and equivocal TBI.

A number of studies have elucidated the role of the S100B protein where mortality is the dependent variable following TBI. A meta-analysis conducted by Mercier et al. (2013) found that, across six studies, a significant positive correlation exists between S100B concentrations and mortality in patients who had sustained moderate or severe TBIs. The researchers found that for mortality, serum threshold values of $>3.0 \mu\text{g/L}$ yielded a specificity of 97%, however the sensitivity at this threshold was only 39%. It was concluded that the capacity for the S100B in the prediction of mortality in TBI patients offers potential utility as part of the decision making process in critical care. They add that there is potential for the biomarker to assist in situations where decisions about level of care are limited in their probabilistic expectations for the patient's prognosis.

Rodriguez- Rodriguez et al. (2012) conducted receiver operator characteristics of serum S100B levels as a predictor of mortality following severe TBI. Their analyses indicated that in a purely “severe” population ($N=55$), by using a cut-off of $.46 \mu\text{g/L}$, sensitivity in predicting mortality was 90% with a specificity of 88.4%. The authors firmly conclude that serum S100B is a sensitive and effective biomarker for the early prediction of mortality following severe TBI. Likewise, as a by-product of their severity prognostic research outline above, Böhmer et al. (2011) reported that S100B levels were higher in patients that died during their study compared with those that survived. Though not the aim of their study, the researchers suggest that a role may exist for the S100B to be utilised in the prediction of potential fatality.

Beyond mortality outcomes, S100B has been investigated in relation to functional outcome for survivors of TBI. In this arm of S100B research, the Glasgow Outcome Scale (GOS; Jennett & Bond, 1975) or the extended version of the scale (GOS-E; Wilson, Pettigrew, & Teasdale, 1998) are by far the most often used measures of outcome. The GOS is a five-point ordinal scale that is completed by the clinician at the follow up consultation. The five outcome values, as determined by the GOS are: 1. Dead; 2. Vegetative State (meaning the patient is unresponsive, but alive); 3. Severely Disabled (conscious but the patient requires others for daily support due to disability); 4. Moderately Disabled (the patient is independent but disabled); and 5. Good Recovery (the patient has resumed most normal activities but may have minor residual problems). The GOS-E, however, is an eight-point ordinal scale whereby the Severely Disabled, Moderate Recovery, and Good Recovery outcome points are stratified into lower and upper bands.

Utilising the GOS-E at one month post-injury, Townend et al. (2002) reported that when adopting 0.48µg/L as a cut-off for “elevated,” patients with elevated levels of serum S100B had a significantly less favourable outcome one month after injury compared to patients who did not have elevated levels of serum S100B. Further, admission S100B levels have been shown to have an inverse relationship with GOS-E outcome at three months (Kleindienst et al. 2010; Walder et al. 2013)) and six months (Raabe & Seifert, 2000) post injury. By comparison to other acute management measures, studies conducted by Woertgen et al. (2002), Nylen et al. (2008), and Wiesmann et al. (2010) all reported that serum S100B concentrations showed a higher correlation to GOS outcome than CT and GCS scores. Similarly, Vos et al. (2010) reported that S100B levels were a stronger predictor of unfavourable outcome at six months post injury than pupillary reactions and GCS score. The researchers conclude by advocating for biomarkers as adjunct procedures to the assessment and management of brain damage after TBI, and speculating that they may enhance prognostic accuracy for clinical outcome variables.

Woertgen et al. (2002) caution that because of its simplicity, the GOS and the GOS-E are considerably non-specific, non-dimensional, and controversial. Di Battista et al. (2013) add that the utility of these scales as dependent variables in outcome research should be questioned, attributable to their limited application for specific clinical symptoms, and their potential for subjective interpretation. Moreover, it could be argued that the unique dimensionality of TBI patients and their outcomes cannot be accurately captured by a seven-point scale. Nevertheless, the GOS and GOS-E do constitute measures of outcome and contribute to top-down theories for biomarker prognoses.

4.4 *S100B and Neuropsychological Outcome*

By stark contrast to the mortality and GOS/GOS-E studies outlined above, relatively little research has been conducted into neuropsychological outcomes following TBI (Watt, Shores, Baguley, Dorsch, & Fearnside, 2006). Inherently, rather than the conventional measures of TBI outcome such as mortality, brain death, and crude ordinal functional impairment, there exists a need for neuropsychological dependent variables to be incorporated into prognostic TBI research, such as cognitive impairment, post-concussion syndrome symptom identification, and deficits in quality of life. Pioneering research conducted by Waterloo, Ingebrigsten and Romner (1997) found that S100B levels were significantly inversely correlated with performances on computerised tests of attention, concentration, and speed of information processing. Although the study's sample size was only seven and the participants had only had "mild" TBIs, the researchers concluded that further research should be conducted to investigate the prognostic merit of S100B for disordered neuropsychological functioning.

Similarly to Waterloo et al (1997), Hermann et al (2001) recruited solely mild TBI patients for their study. The research design involved administering a battery of neuropsychological tests six months after TBI. The study found that serum levels of S100B on admission were strongly correlated with poor performance on measures of attention, discrimination, and memory. Despite clear correlations, however, Hermann et al.'s study had a very small sample size of 39, and used measures of neuropsychological functioning that are somewhat antiquated by today's standards. Similarly, using an Australian sample of 23 severe TBI patients, Watt et al. (2006) investigated the ability of S100B to predict the extent of neuropsychological impairment following injury. Participants were subjected to a neuropsychological

battery following their injury, however unlike Hermann et al.'s study, participants were given this battery within two weeks of emerging from post-traumatic amnesia. Watt et al.'s battery involved the administration of a full WAIS-R (Wechsler, 1981), as well as assessments of auditory learning memory, verbal fluency, attention, concentration, and speed of information processing. The researchers suggest that their results indicate that post-traumatic serum concentrations of S100B not only reflect brain injury severity, but also relate to deficits in neuropsychological functioning.

In reviewing Watt et al.'s findings, it is important to note that the conclusions generated by the study are applicable to the immediate outcome of TBI. However, as mentioned earlier in this review, the most variability in TBI patients' neuropsychological outcome is across the first six months of post-injury recovery. Further, their study utilised a control group in order to investigate between-group differences in functioning, rather than examining magnitude of impairment at an individual level. As such, methodological manipulations are indicated for researching cognitive impairment following TBI in order to more fully elucidate the prognostic utility of a proposed independent variable. With reference to dependent variables, it is pertinent to ensure that neuropsychological consultation is short and uses a minimum number of simple measurement instruments (Van Baalen et al., 2006).

Beyond direct assessments of cognitive functioning, other instruments have been utilised for investigating S100B and neuropsychological outcome. The Rivermead Post-Concussion Symptoms Questionnaire (RPQ; King, Crawford, Wenden & Wade 1995) is an instrument that is often used to measure the presence of PCS-related symptoms, with persistence and severity compared to before the injury. Savola and Hillborn (2003) found that levels of serum S100B were correlated with

RPQ scores at two and six weeks following injury. Likewise, Begaz et al. (2006) showed that patients who have suffered a mild TBI exhibit a correlation between levels of serum S100B, and sub-acute identification with symptoms of post-concussion syndrome. Beyond the acute stage, Bazarian, Blyth, Zemlan and Stigman (2006) reported that serum S100B levels were correlated with RPQ scores at three months post-injury. Conversely, in a paediatric study, S100B did not predict which children who had sustained a TBI would develop PCS or the severity of their symptoms (Babcock, Byczkowski, Wade, Ho, & Bazarian, 2013).

In an investigation of symptom identification, Sojka, Stålnacke, Björnstig and Karlsson (2006) found an association between serum levels of S100B and a number of symptoms related to stress disorders. Somatically, Kondziolka (2013) reported that TBI patients with higher levels of S100B more often complained of nausea and vomiting. From an organisational and rehabilitative perspective, Stranjalis et al. (2004) found that probability for returning to work following mild head injury was inversely correlated with levels of S100B. More recently, Egea-Guerrero et al. (2013) reported that serum S100B levels were correlated with time taken to return to work, and the reappearance of residual somatic symptoms six months post injury. The authors highlight the potential utility of the biomarker to be used in predicting the development of long-term sequelae for TBI patients' quality of life.

Chapter 5

Rationale for the Current Research

The aim of the present thesis was to investigate the prognostic utility for serum S100B following TBI, with specific emphasis on neuropsychological functioning as the definable outcomes. Using a large prospective sample of TBI patients, across each classification of severity, this longitudinal population study aimed to examine the relationships that exist between S100B and current acute measures of TBI severity. Further, this thesis aimed to elucidate the ability for S100B to make prognoses for duration of post-traumatic amnesia, severity of cognitive impairment, presence of symptoms of post-concussion syndrome, and deficits in domains that are associated with quality of life.

In Study One, this thesis aimed to identify potential relationships between serum levels of S100B and the current acute measures for classifying TBI severity – namely the GCS, and the WPTA. Further, Study One aimed to quantify the accuracy that S100B holds in prognosing duration of post-traumatic amnesia as measured by the WPTA, and whether this prognostic accuracy is superior to that of GCS scores. In Study Two, this thesis aimed to quantify the variability demonstrated in cognitive impairment at two months post TBI and the ability for S100B to prognose such impairment. Rather than using between-group comparisons with healthy controls, Study Two defined impairment as the magnitude of discrepancy between post-injury functioning and quantified estimates of premorbid functioning. Lastly, in Study Three, this thesis aimed to examine deficits in domains of quality of life and the severity of post-concussion syndrome symptomatology at six months post injury, and to investigate the ability for S100B to prognose such deficit and symptom severity.

Chapter 6

Study One: Serum S100B and Existing Diagnostic Variables (T_0)

6.1 *Aims and Hypotheses*

The focus of Study One: T_0 was on the diagnosis and management of TBI immediately following injury (i.e., 0 days post TBI). In this context, Study One aimed to investigate the diagnostic, categorical, and operational utility of a single collection of serum S100B, collected as soon as possible following injury. To elucidate this utility, Study One aimed to identify potential relationships between serum levels of S100B and the current acute measures for classifying TBI severity – namely the Glasgow Coma Scale, and the Westmead Post-Traumatic Amnesia Scale.

With an emphasis on prognostic utility, Study One further aimed to quantify the accuracy that S100B holds in predicting duration of post-traumatic amnesia as measured by the WPTA and whether this prognostic accuracy is superior to that of GCS scores. In short, the aim was to quantify whether the number of days that a patient will spend in post-traumatic amnesia can be predicted from their acute presentation.

As discussed in the preceding literature review, the presence of S100B has been shown to discriminate TBI from non-TBI patients (Bohmer et al., 2011; Matek et al., 2012), and also to discriminate severity of injury within TBI populations, as measured by the GCS (Naeimi et al., 2006; Savola et al., 2004). Serum S100B levels have also found to be significantly higher in patients who deteriorate to brain death compared to those who do not (Dimopolou et al., 2003). A number of imaging studies have also found a relationship between serum levels of S100B and the presence of secondary pathologies following injury (Bazarian et al., 2013; Cervellin et al., 2012; Herrman et al., 2001; Morochovic et al., 2009).

Beyond injury presence and severity, the literature has shown a significant inverse relationship between serum levels of S100B immediately after injury, and depth of coma as measured by the GCS (Herrmann et al., 2001; Korfias et al., 2007; Watt et al., 2006). Further, Watt et al. (2006) reported a strong positive relationship between S100B and duration of post-traumatic amnesia as measured by the WPTA. Based on the associated literature, the hypotheses for Study One: T₀ were:

- H₁: S100B levels would be significantly negatively correlated with GCS scores, i.e., the deeper the coma, the higher the level of S100B.
- H₂: S100B levels would be significantly positively correlated with WPTA scores, i.e., the higher the level of S100B, the longer the patient will experience post-traumatic amnesia.
- H₃: As per H₂, the relationship between S100B and WPTA would be such that length of WPTA can be algorithmically predicted by serum levels of S100B.

6.2 Method

6.2.1 Participants

Adult patients who presented to the Royal Hobart Hospital following a TBI were approached to participate in this study. Participants were provided with an information sheet for the study and a participant consent form. When patient consent was not possible due to coma or sedation, consent was sought from the patients' next of kin. The patient's information sheet, patient's consent form, relative's information sheet, and relatives consent form used in this study can be found in Appendix B. No participants received payment for participation in this study.

In total, 127 adult TBI patients were recruited as participants for this study. The participant pool consisted of 96 (75.59%) males and 31 (24.41%) females. The

age range for participant recruitment was $18 \leq 80$, and the mean age of the participants was 41.23 years with a standard deviation of 16.66 years. The sex and age ratios in this study are consistent with Australian population epidemiology estimations (Fortune & Wen, 1999).

This study approached all patients presenting with TBI to participate, and as such, various aetiologies of injury were evident within the participant pool. Table 6.1 below displays the TBI aetiologies of the study's participants.

Table 6.1
Aetiologies of Injury within the Participants

Mechanism	<i>n</i>	Percentage
Motor vehicle accident	42	33.07%
Fall from height	23	18.11%
Assault	18	14.17%
Motorbike accident	12	9.45%
Mechanical fall	9	7.09%
Falling object	5	3.94%
Pedestrian versus car	4	3.15%
Horse riding injury	4	3.15%
Bicycle accident	3	2.36%
Quadbike accident	3	2.36%
Sports injury	2	1.57%
Skateboard	1	0.79%
Projectile	1	0.79%

Note. $N = 127$

Participants who had a blood sample taken immediately following their injury, received a GCS score on admission, and received WPTA testing during their admission (greater than one day) were included in analysis at the T_0 time point.

From the initial participant pool of 127 TBI patients, 79 participants met the inclusion criteria for the T_0 time point. Table 6.2 displays the severity categorisation frequencies (as defined by Jennett and Teasdale, 1981) on the GCS and WPTA for the T_0 participants.

Table 6.2
Severity Categorisation Frequencies for Glasgow Coma Scale and Westmead Post-Traumatic Amnesia Scale

Glasgow Coma Scale	Definition	Frequency
Mild	GCS = 13<15	55 (69.62%)
Moderate	GCS = 9<12	7 (8.86%)
Severe	GCS = 3<8	17(21.52%)
Westmead Post-Traumatic Amnesia Scale	Definition	Frequency
Severe	WPTA = 1 to 7 days	39 (49.37%)
Very Severe	WPTA = 8 days +	40 (50.63%)

Note. N = 79.

6.2.2 Materials

6.2.2.1 Serum S100 Calcium Binding Protein B (S100B) Assay

Following injury, blood tests are performed on patients as part of routine acute medical management of TBI. For the participants of this study, S100B assay analyses were conducted on serum aliquots taken from blood samples taken as early as possible after injury. The post-injury sample time for this study's population had a mean of 2.8 hours, and a standard deviation of 3.4 hours.

Serum samples were analysed using commercially available LIAISON® S100 assay kits. This kit is a two-step quantitative chemiluminescence sandwich immunoassay which uses directly coated magnetic particles and an isoluminol derivative. Blood samples taken from patients were centrifuged at 800-1000 rotations per minute for 10 minutes. Aliquots were taken from the resulting separated serum and frozen at -20° Celsius for analysis at a later date. Batch analyses of all collected samples were then conducted prior to the oldest samples becoming six months post injury.

The S100B assays performed in this study were conducted on a commercially available LIAISON® S100 analyser kit, following a two-site chemiluminescent immunoassay (CLIA) procedure. The LIAISON® S100 analyser kit is stable at 2-8° Celsius until its expiry date and can be used for up to two weeks after being opened.

The minimum sample volume required for detection of S100B determination is 100µL, and the procedure is capable of detecting S100B concentrations of between .02µg/L to 30µg/L.

The LIAISON[®] S100 assay manual cites that 95 percent of healthy individuals have serum S100B levels <.15µg/L. Assay analyses were conducted on samples taken from 15 non-TBI controls (serum samples from hospital presentations with confirmed non-TBI). The non-TBI controls in this study had a mean of 0.097µg/L, and a standard deviation of .052µg/L, and the participants' mean S100B level was 3.17µg/L, with a standard deviation of 5.72µg/L.

All S100B analyses in this study were conducted by an independently hired medical scientist at the Royal Hobart Hospital department of pathology who was blinded to the names of the participants and the nature of their injuries.

6.2.2.2 *The Glasgow Coma Scale (GCS)*

The GCS (Teasdale and Jennett, 1974) is a brief objective rating scale that is used to assess a patient's level of consciousness. Following injury, the evaluator tests the patient's response to three domains, namely: eye opening (scale of 1-4); verbal communication (scale of 1-5); and, motor response to pain (scale of 1-6). A GCS total score is the sum of the highest score in each of the three domains. As such, GCS total scores range from 3 to 15. Table 6.3 displays the scoring criteria for each of the GCS domains.

Table 6.3
Scoring Criteria for the Glasgow Coma Scale

Eye	Patient Response	Score
	Does not open eyes	1
	Opens eyes to pain (e.g., when pinched)	2
	Opens eyes when asked	3
	Opens eyes normally	4
Verbal	Patient Response	Score
	Makes no noise	1
	Unintelligible noise (e.g., groans)	2
	Nonsensical speech	3
	Coherent speech, yet confused/disoriented	4
	Oriented, correct conversation	5
Motor	Patient Response	Score
	No motor response to pain	1
	Extension (extensor posturing) to pain	2
	Abnormal flexion (flexor posturing) to pain	3
	Withdraws/pulls away from pain	4
	Localisation to pain	5
	Follows simple motor commands	6

Following TBI, the severity of a patient's depth of consciousness is classified based on their GCS total score as being Mild (GCS: $15 \geq 13$), Moderate (GCS: $12 \geq 9$), or Severe (GCS: ≤ 8). Patients' level of consciousness is routinely monitored across triage and admission to quantify recovery of consciousness. For this study's analyses, each patient's earliest recorded GCS total score is used. These evaluations are often conducted on scene by ambulance officers, prior to the patient's hospital admission. The GCS protocol can be found in Appendix C.

6.2.2.3 Westmead Post-Traumatic Amnesia Scale (WPTA)

The WPTA (Marosszesky, Ryan, Shores, Batchelor, and Marosszesky, 1997) is a standardised scale that is regularly used in inpatient settings to objectively measure post-traumatic amnesia following TBI. In this context, post-traumatic amnesia is operationally defined as the period of time where new memories are not consolidated, and not merely the time between the injury and the patient's first subjectively recalled memory. Administration of the WPTA involves asking the

patient seven orientation questions followed by five recognition/memory questions.

The questions for the WPTA are displayed below in Table 6.4.

Table 6.4

Administered Questions for the Westmead Post-Traumatic Amnesia Scale

Item	Question
1	How old are you?
2	What is your date of birth?
3	What month are we in?
4	What time of day is it? (morning, afternoon, or night)
5	What day of the week is it?
6	What year are we in?
7	What is the name of this place?
8	Have you seen my face before?
9	What is my name?
10, 11, & 12	What were the three pictures that I showed you yesterday?

A patient is said to be out of post-traumatic amnesia if they can achieve a perfect score on the WPTA Scale for three consecutive days. Post-traumatic amnesia is deemed to have ended on the first of the three consecutive days of perfect recall. As per the WPTA protocol, the first day of perfect recall is used for this study's analyses. The severity of a patient's post-traumatic amnesia is classified based on their WPTA score as being Moderate (WPTA: < 1 days), Severe (WPTA: $1 \leq 7$ days), and Very Severe (WPTA: > 8 days). The WPTA protocol can be found in Appendix C.

6.2.3 Procedure

This study received Medical Ethics Approval by the Tasmanian Health and Human Research Ethics Committee (Ref. No H0010420; Appendix A).

Ambulance reports (contained in each patient's medical records) were reviewed to record time of injury. For this study, the "call received" time noted on the ambulance report was used as the quantifiable approximation of actual time of injury.

In study one (T_0), aliquots of serum were taken from participants' earliest blood sample that had been collected during routine medical care, and the time of blood collection was recorded to quantify the collection time post-injury. Batch (group) analyses of patient aliquots were performed to determine serum S100B levels for each participant.

GCS scores for each participant were recorded from their first evaluation post-injury. As mentioned in the *Materials* section, these initial GCS evaluations were often conducted on scene by ambulance officers prior to the patient's admission to hospital.

WPTA scores were collected from review of participants' medical records. As WPTA screenings are regularly conducted as part of inpatient management of TBI, these evaluations were not conducted by the present researcher – rather, they were undertaken by occupational therapists and nurses involved in the patient's care.

6.2.4 Design and Analyses

Patients' S100B values and depth of coma (as measured by the GCS) constituted the independent variables, and duration of post-traumatic amnesia (as measured by the WPTA) constituted the dependent variable. Two-tailed bivariate correlations with Pearson coefficients were conducted to explore interrelationships between S100B and the existing diagnostic variables (GCS and WPTA).

The ability for S100B to predict duration of post-traumatic amnesia was examined using robust linear regression, and subsequent regressions investigated whether the proportion of variance accounted for could be improved by including GCS scores in the model. Jarque-Bera analyses were conducted on the skewness and kurtosis of each model to test for normality of distribution, and Breusch-Pagan

heteroscedasticity analyses were conducted on each model to investigate whether the variance (actual versus predicted) in the residuals were dependent on the value of the dependent variable (duration of PTA).

Diagnostic and agreement statistics were then conducted to compare the respective sensitivity and specificity of S100B and GCS in identifying future “Very Severe” PTA (as classified by the WPTA), and Receiver Operating Characteristics were conducted to determine the area under the curve (AUC) for each independent variable as an indicator of “Very Severe” PTA.

6.2.5 Statistical Software and Manipulations

6.2.5.1 Correlations and Multiple Linear Regressions

Correlation and regression analyses were conducted utilising SPSS® Version 21 (IBM Corp., 2012). Further, this software was used to generate predicted values and residuals for each regression equation. Descriptive analyses were conducted to derive the skewness and kurtosis of the residuals for each equation in order to facilitate Jarque-Bera tests for normality.

6.2.5.2 Tests for Normality

Jarque-Bera tests for normality were computed by authoring formulas in Excel 2010 (Microsoft, 2010) for each equation. χ^2 analyses were then conducted on the resulting Jarque-Bera values. The algorithm used for deriving Jarque-Bera values is depicted below in *Equation 6.1*, whereby n is the number of observations and S and K are the skewness and kurtosis of the residuals for the given equation.

$$JB = n \times (S^2/6 + K^2/24) \quad (Eq. 6.1)$$

6.2.5.3 Regression Equation Outliers

For each regression equation, outliers were removed by satisfying two criteria for case removal. First, as per Tabachnick and Fidell (2013), any cases that had a standardised residual value of more than 3.3 or less than -3.3 were identified as an outlier. Secondly, as per Field (2013), leverage values were calculated to determine the influence of each case's outcome on the model, and cases were identified as outliers if the leverage value was greater than $3(k+1)/n$ whereby k is the number of predictors and n is the number of observations. Only cases that were identified as outliers by both methodologies were excluded from analysis.

6.2.5.4 Heteroscedasticity

Breusch-Pagan tests for heteroscedasticity were conducted using SPSS[®] Version 21 to calculate the squared residuals, and then to compute a variable equal to the squared residual divided by the sum of the squared residuals, divided by the number of observations. Regression analyses were then conducted using the computed variable against the predicted values. The sum of squares resulting from these regression equations were then entered into Excel 2010 formulas to derive Breusch-Pagan values, and to conduct post-hoc χ^2 analyses of the Breusch-Pagan value to determine statistical significance.

6.2.5.5 Robust Regression Analyses

For any equations that tested as significant for heteroscedasticity, post hoc robust regression analyses (using a macro authored by Hayes & Cai, 2007) were conducted in SPSS[®] Version 21 to determine the significance of the coefficients to the adjusted standard errors.

6.2.5.6 Receiver Operating Characteristics and Diagnostic and Agreement Statistics

SPSS[®] Version 21 was used to conduct Receiver Operating Characteristics in order to determine the area under the curve (AUC) for each independent variable as an indicator (or criterion) for any classification. Cut-off points were determined for each independent variable to facilitate respective Diagnostic and Agreement statistical analyses. Diagnostic and Agreement statistics reported in this study were conducted using DAG_Stat (Mackinnon, 2000), which is a freely available Excel worksheet used to determine statistical sensitivity and specificity. Positive- and Negative-Predictive Values were not calculated, as empirically-derived base-rates for each classification (or condition) could not be determined.

6.3 Results

6.3.1 Classifications and Diagnostic Correlations

The participants classified as having “Moderate” and “Severe” GCS scores had significantly higher S100B levels than those classified as “Mild,” $t(24.501) = 2.739, p < .01$, and the participants classified as having “Severe” GCS scores had significantly higher S100B levels than those classified as “Mild” and “Moderate” $t(17.612) = 2.679, p < .05$. Two-tailed Pearson’s correlations were conducted between serum S100B levels, depth of coma (GCS), and duration of post-traumatic amnesia (WPTA). As displayed in Table 6.5, serum S100B levels were significantly correlated with depth of coma (GCS) and duration of post-traumatic amnesia (WPTA).

Table 6.5
Correlations between Serum S100B, Glasgow Coma Scale, and Westmead Post-Traumatic Amnesia

	S100B	GCS	WPTA
S100B	-	-.419**	.415**
GCS	-	-	-.550**
WPTA	-	-	-

Note. ** $p < .01$, two-tailed; $N = 79$.

6.3.2 Predicting Duration of Post-Traumatic Amnesia

Following removal of three outlier cases, serum S100B levels significantly predicted duration of post-traumatic amnesia as measured by the WPTA, $R^2 = .361$, $F(1, 75) = 41.845$, $p < .001$. The Jarque-Bera test for normality of the first model was not significant $\chi^2(2, N=76) = 5.150$, $p=.076$, and the algorithm for this model is presented in *Equation 6.2*.

$$\text{WPTA} = (\text{S100B} \times 1.627) + 8.363 \quad (\text{Eq. 6.2})$$

Depth of coma as measured by the Glasgow Coma Scale was added to this equation as an additional independent variable. Depth of coma significantly added to the variance accounted for by *Equation 6.2*, $R^2 = .474$, $\Delta R^2 = .113$, $F(2, 75) = 32.955$, $p < .001$. The Jarque-Bera test for normality of the second model was significant $\chi^2(2, N=76) = 10.447$, $p < .001$, and the algorithm for this model is presented in *Equation 6.3*.

$$\text{WPTA} = (\text{S100B} \times 1.188) + (\text{GCS} \times -1.302) + 25.420 \quad (\text{Eq. 6.3})$$

6.3.3 Heteroscedasticity of T_0 Regression Models

Breusch-Pagan heteroscedasticity analyses were conducted on the two models to investigate whether the variance (actual versus predicted) in the residuals of each model were dependent on the value of the dependent variable (duration of PTA).

A Breusch-Pagan test conducted on *Equation 6.2* (S100B predicting WPTA) was significant, $\chi^2(1, N=76) = 21.211, p < .001$. Robust regression analysis, however, revealed that S100B remained a significant predictor of WPTA, despite heteroscedasticity of the model ($\beta=1.623, p < .05$). A post-hoc bivariate Pearson's correlation revealed a significant moderate correlation between duration of PTA and magnitude of error in the residuals (.542, $p < .001$).

The Breusch-Pagan test conducted on the *Equation 6.3* (S100B and GCS predicting WPTA) was significant, $\chi^2(1, N=76) = 33.055, p < 0.001$. However, a significant moderate correlation still remained between duration of PTA and the magnitude of error in the residuals (.479, $p < .001$). Figures 6.1 and 6.2 display the residual error across duration of PTA for each equation.

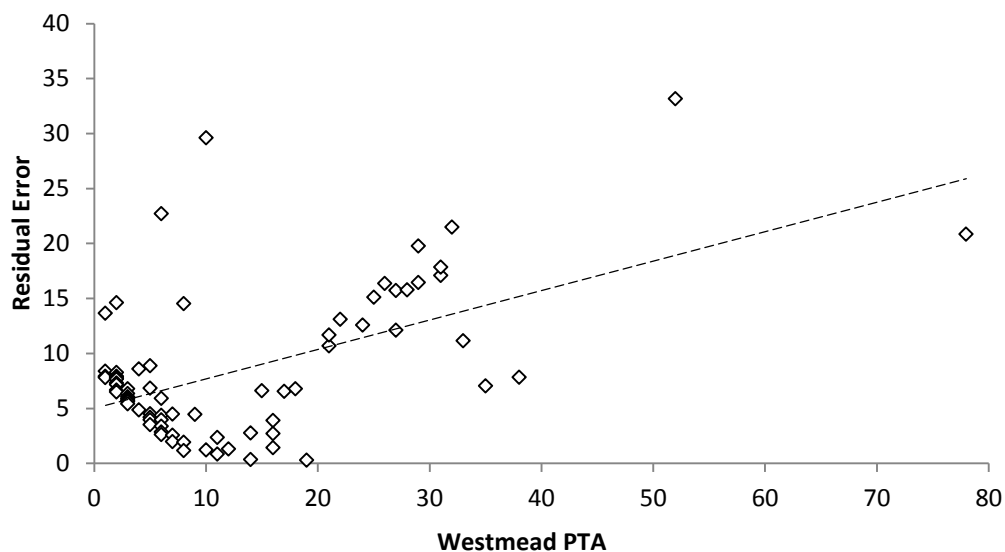


Figure 6.1 The residual error for *Equation 6.2* across duration of post-traumatic amnesia

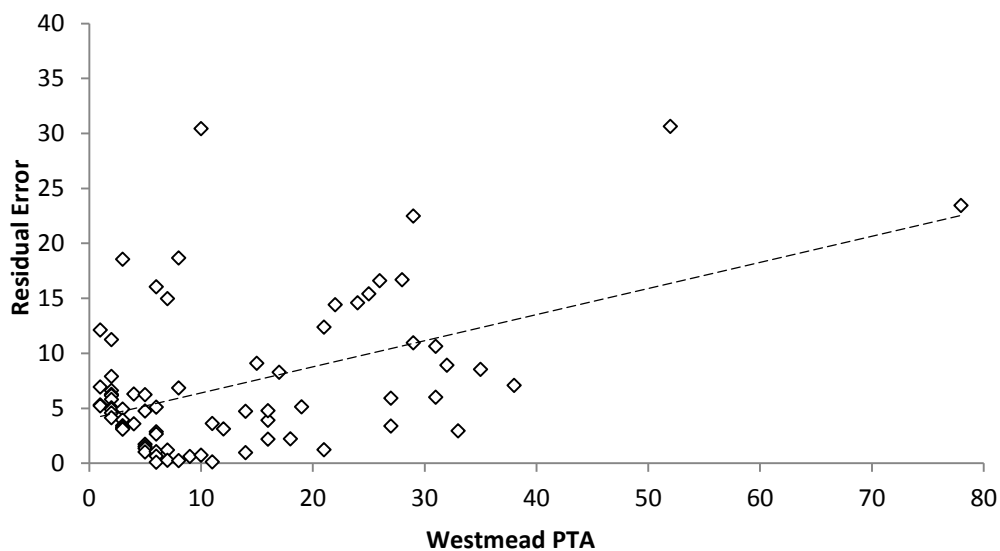


Figure 6.2. The residual error for Equation 6.3 across duration of post-traumatic amnesia.

By restricting the range of duration of post-traumatic amnesia estimations to ≤ 30 days, there was no significant correlation between duration of PTA and magnitude of error in the residuals for either Equation 6.2 (.012, $p=.921$), or Equation 6.3 (.169, $p=.169$). Figures 6.3 and 6.4 display the residual error across the first 30 days of PTA for each equation.

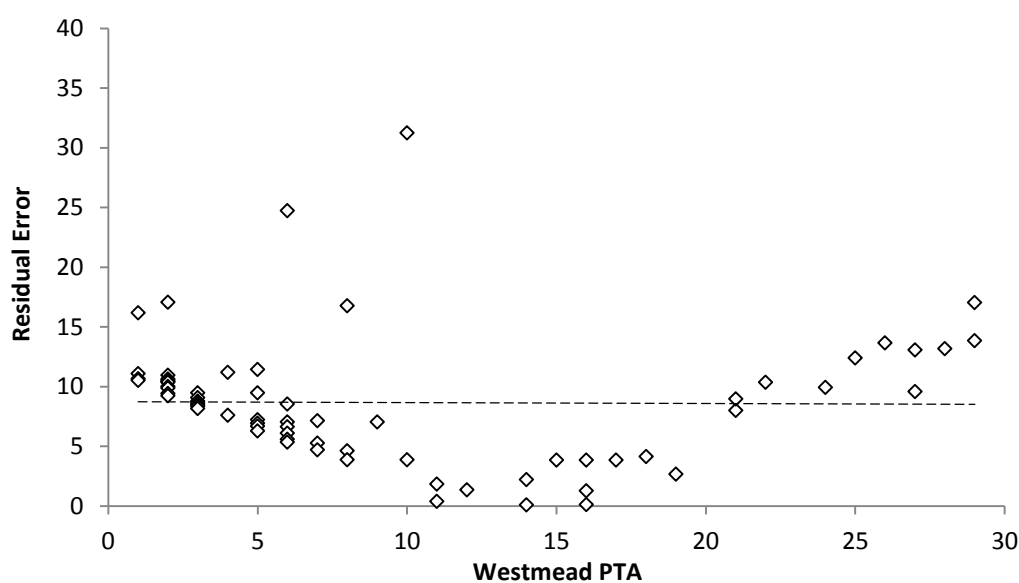


Figure 6.3. The residual error for Equation 6.2 across 30 days of post-traumatic amnesia.

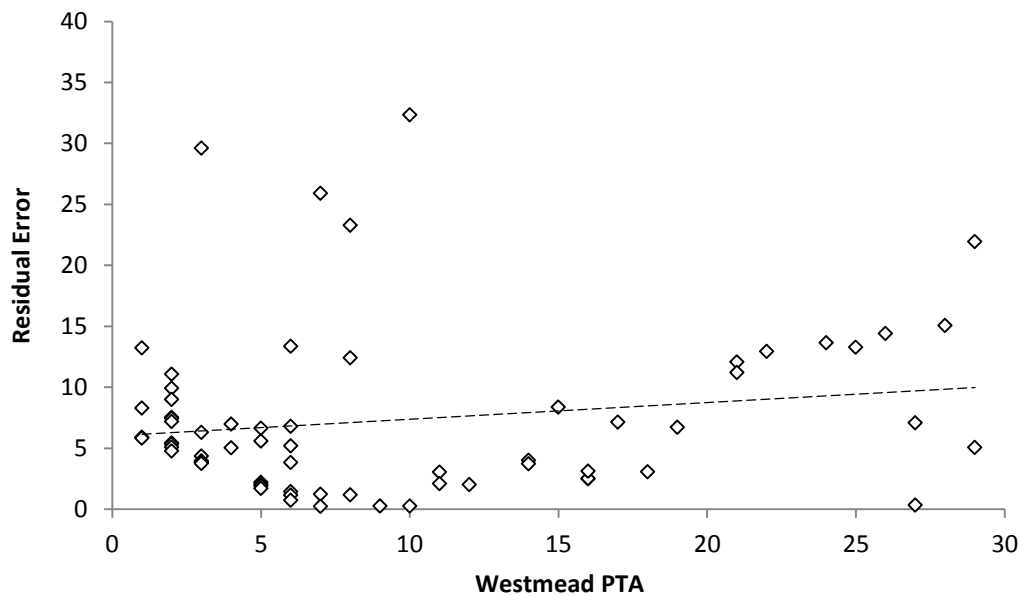


Figure 6.4. The residual error for Equation 6.3 across 30 days of post-traumatic amnesia.

6.3.4 Sensitivity and Specificity of Predicting “Very Severe” Post-Traumatic Amnesia

40 of the 79 T_0 participants were classified in the “Very Severe” range of post-traumatic amnesia as measured by the WPTA (≥ 8 days). Receiver operating characteristics conducted on S100B and “Very Severe” PTA indicated an AUC of .772. The S100B cut off point with the highest statistical efficiency (also known as “correct classification rate”) for identifying “Very Severe” post-traumatic amnesia was $0.97\mu\text{g/L}$. Cohen’s Kappa analysis of this cut off point indicated moderate agreement for classification, $\kappa = .493$, $p < .001$. The participants classified as exhibiting “Very Severe” PTA had significantly higher S100B levels, $t(48.330) = -2.914$, $p < .01$.

Receiver operating characteristics conducted on GCS and “Very Severe” PTA indicated an AUC of .804. The GCS cut off point with the highest statistical efficiency for identifying “Very Severe” post-traumatic amnesia was 12 (this is also

the empirical/clinical cut off for “Moderate” TBI). Cohen’s Kappa analysis of this cut off point also indicated moderate agreement for classification, $\kappa = .471, p < .001$. The participants classified as exhibiting “Very Severe” PTA had significantly lower GCS scores, $t(40.003) = -4.637, p < .01$.

The Receiver Operator Characteristics for S100B (AUC = .772) and GCS (AUC = .804) in identifying “Very Severe” range post-traumatic amnesia are depicted below in Figure 6.5

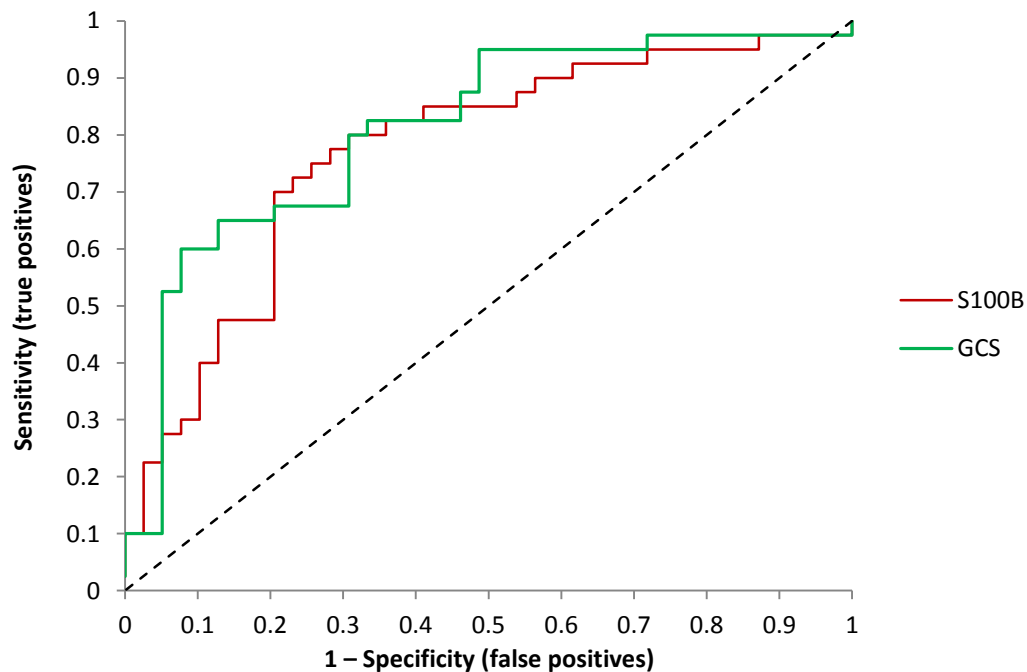


Figure 6.5 Receiver Operating Characteristics of S100B and GCS with “Very Severe” post-traumatic amnesia.

The diagnostic and agreement statistics for S100B using 0.97µg/L and GCS using 12 as respective cut-offs for identifying “Very Severe” range post-traumatic amnesia are displayed below in Table 6.6.

Table 6.6

Diagnostic and Agreement Statistics for Identifying “Very Severe” PTA

	S100B		GCS	
Sensitivity	.775	(.615 – .892)	.525	(.361 – .685)
Specificity	.718	(.551 – .850)	.949	(.827 – .994)
Efficiency	.747	(.636 – .838)	.734	(.623 – .827)
False Positive Rate	.282	(.150 – .449)	.051	(.006 – .173)
False Negative Rate	.225	(.108 – .385)	.475	(.315 – .639)
Misclassification Rate	.253	(.162 – .344)	.266	(.173 – .501)

Note. 95% confidence intervals are displayed in parentheses. S100B cut off $\geq 0.97\mu\text{g/L}$; GCS cut off ≤ 12 .

Figures 6.6 through 6.9 on the following pages illustrate the respective sensitivity and specificity of S100B and GCS in identifying “Very Severe” post-traumatic amnesia ($\text{WPTA} \geq 8$ days). The respective cut-offs for each independent variable (established above) are depicted in each plot.

Figures 6.6 and 6.7 below plot the sensitivity and specificity of S100B in identifying “Very Severe” post-traumatic amnesia (WPTA ≥ 8 days), the cut off level with the highest efficiency (.97 $\mu\text{g/L}$) is depicted in the plot.

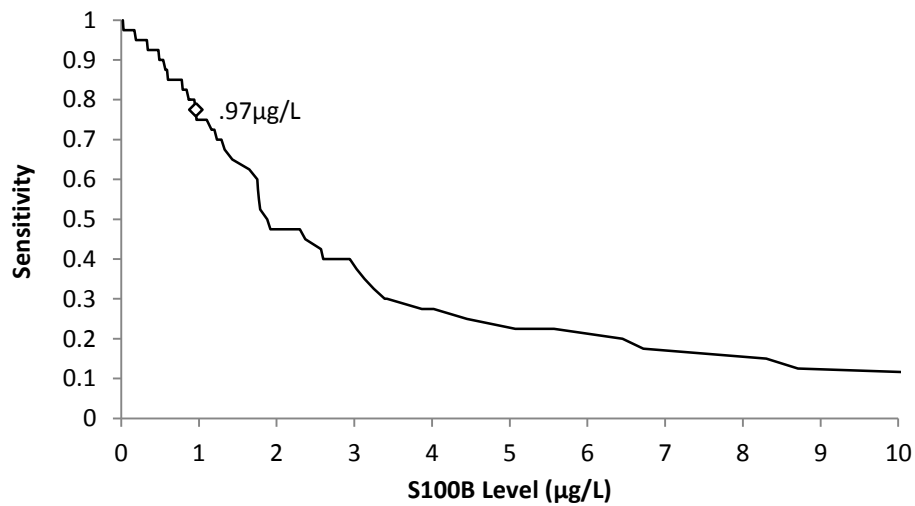


Figure 6.6. The sensitivity of serum S100B levels in identifying “Very Severe” post-traumatic amnesia

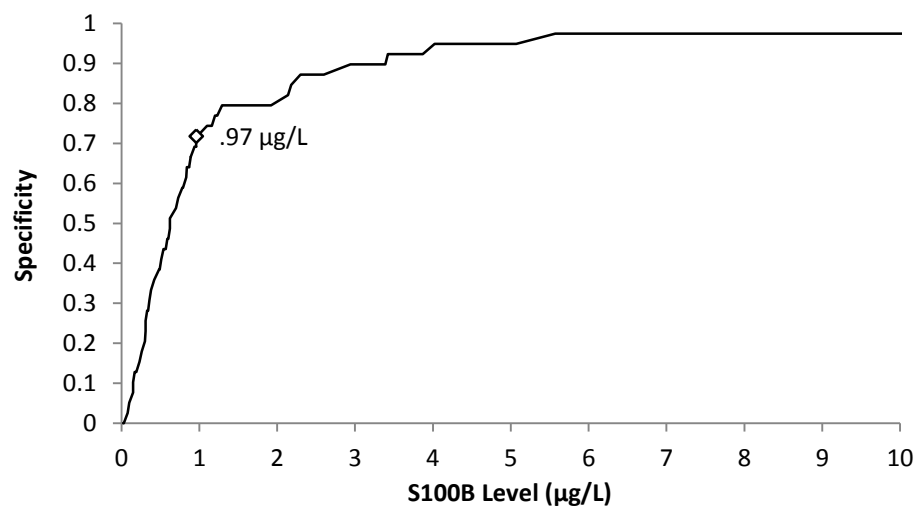


Figure 6.7. The specificity of serum S100B levels in identifying “Very Severe” post-traumatic amnesia

Figures 6.8 and 6.9 below plot the sensitivity and specificity of depth of coma in identifying “Very Severe” post-traumatic amnesia, the cut off level with the highest efficiency (12) is depicted in the plot.

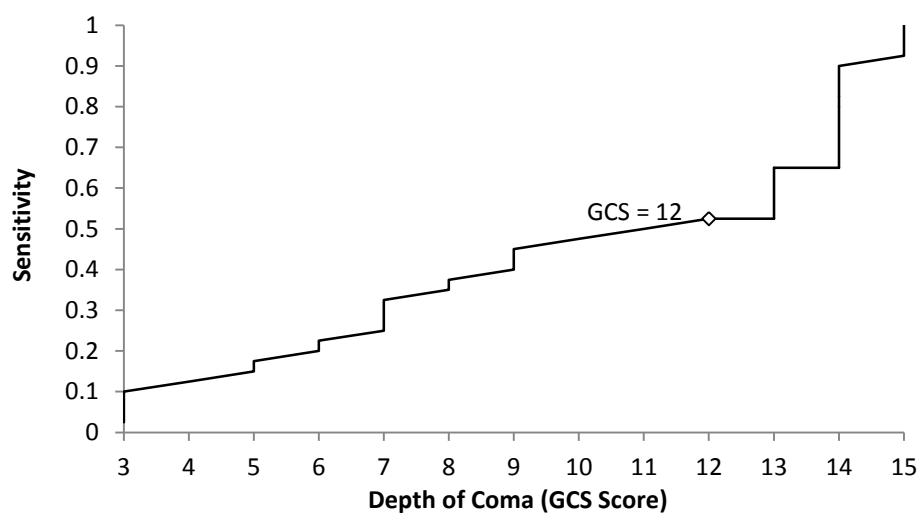


Figure 6.8. The sensitivity of GCS scores in identifying “Very Severe” post-traumatic amnesia (≥ 8 Days).

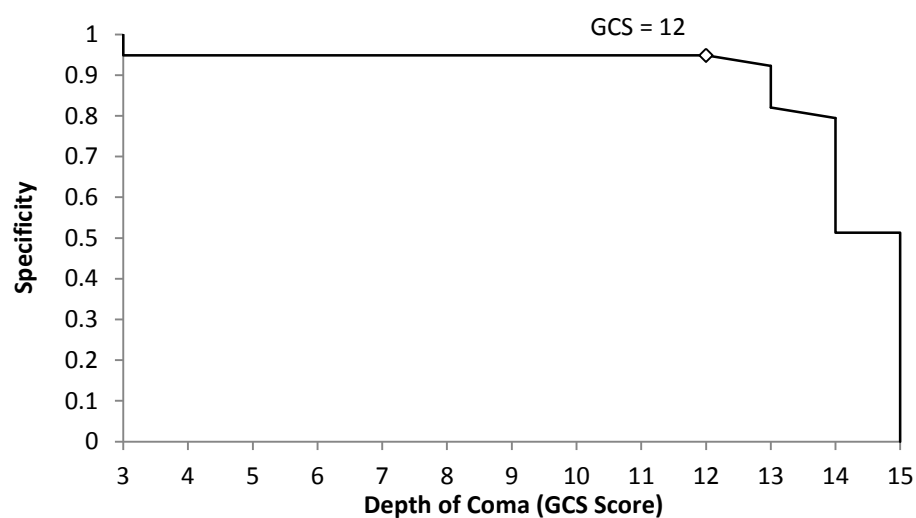


Figure 6.9. The specificity of GCS scores in identifying “Very Severe” post-traumatic amnesia (≥ 8 Days).

6.4 Discussion

This study investigated the utility of serum S100B with reference to the diagnosis and management of TBI immediately following injury. More specifically, the present study elucidated the diagnostic, categorical, and operational utility of a single collection of serum S100B by identifying statistical relationships between serum levels of S100B and the current acute measures for classifying TBI severity – namely the Glasgow Coma Scale (GCS), and the Westmead Post-Traumatic Amnesia Scale (WPTA).

The associated literature, discussed in the preceding literature review, indicates that the presence of S100B can be used to discriminate TBI from non-TBI patients (Bohmer et al., 2011; Matek et al., 2012), and also to discriminate the severity of injury within TBI populations (Naeimi et al., 2006; Savola et al., 2004). Inherently, a statistical relationship could be hypothesised between level of serum S100B in the peripheral system immediately following injury, and the magnitude and severity of the injury itself.

6.4.1 *S100B and Depth of Coma*

In alignment with the aforementioned assumption, this study hypothesised a significant inverse correlation between serum S100B levels and GCS scores. Put simply, it was hypothesised that the higher the level of S100B, the deeper the coma following injury. This hypothesis was supported by the data, in that a significant negative correlation was found between serum S100B levels and GCS scores ($r = -.419$). This finding is comparable to those of Herrmann et al. (2001), Watt et al. (2006), and Korfias et al. (2007), who found significant inverse relationships between S100B and GCS of $r = -.570$, $-.470$ and $r = -.331$ respectively.

In addition to the inverse correlation found between serum S100B levels and GCS scores, this study also illustrated that S100B was able to categorically discriminate between mild, moderate, and severe depths of coma as measured by the GCS. This finding extends the preliminary results reported by Naeimi et al. (2006) who found that S100B was able to discriminate between severe head injuries and mild and moderate injuries combined. However, this significant categorical result contrasts with the investigation conducted by Beers et al. (2007). In their study of 15 paediatric patients, the researchers reported that S100B did not differ significantly across GCS categories. It should be noted that the present research utilised a significantly larger participant population, and the nature of the participant pool's injuries was markedly more severe. The discrepancies between the findings of the current study and Beers et al.'s is likely to be attributable to these two constituents of methodological design.

6.4.2 *S100B and Post-Traumatic Amnesia*

As per the assumed relationship between level of S100B and the severity of injury, this study hypothesised a significant correlation between serum S100B levels and WPTA scores. Put simply, it was hypothesised that the higher the level of S100B, the longer the patient would remain in post-traumatic amnesia following their injury. This hypothesis was supported by the data, in that a significant correlation was found between serum S100B levels and WPTA scores ($r = .415$). This finding is comparable to that of Watt et al. (2006) who found a significant relationship between initial levels of S100B and WPTA of $r = -.590$. To date, Watt et al.'s study is the only published research to investigate the relationship between S100B and any quantifiable duration of post-traumatic amnesia.

It was further hypothesised in current study that WPTA scores could be algorithmically predicted by serum levels of S100B. It is argued that this final hypothesis held the strongest pertinence towards clinical utility and translational modelling. Being that duration of post-traumatic amnesia is the best indicator of the extent of cognitive and functional deficits that follow TBI (Khan et al., 2003), the ability to predict the days or weeks of post-traumatic amnesia that a patient is likely to endure, within hours of their injury, is of clear utility to the treating physicians and to the carers and family of the patient. S100B meets these criteria, with the addition of being unaffected by blood alcohol – unfortunately, depth of coma and duration of loss of consciousness cannot claim this desirable attribute.

In the present study, serum S100B levels were shown to be able to predict duration of post-traumatic amnesia as measured by the WPTA. Although S100B in isolation was only capable of accounting for 36.1% of the variance in WPTA outcome, including the patient's GCS score (also attainable immediately following injury) to the algorithm resulted in a significant increase of variance accounted for. The results showed that any prognoses for post-traumatic amnesia beyond 30 days held unacceptable clinical confidence intervals, indicating the longer the prognosed post-traumatic amnesia, the larger the margin for error. However, by restricting the estimated range to less than 30 days, no correlation remained between the duration of post-traumatic amnesia and magnitude of error in the residuals. It could be reasonably argued that the unacceptable statistical error post 30 days is clinically irrelevant, and that the prediction of which patients will recover within the first few days and which will not is of the most pertinence to the medical management of TBI patients.

6.4.3 *Conclusions*

The above findings contribute to the empirical research concerned with validation of S100B in the medical management of TBI. Further, these results extend the associated literature by investigating biomarker utility with a clinical variable that is currently used in the treatment and management of TBI – namely the WPTA. From a translational perspective within the context of the acute setting, it can be argued that for a biomarker to be clinically validated, it must be shown to have utility beyond mortality. For the treating clinicians, it is the medical management of the survivors of TBI which is of most relevance.

Inarguably, the most pertinent finding from the current study was that S100B was able to statistically classify which patients would endure “Very Severe” post-traumatic amnesia (greater than one week following injury) as measured by the WPTA. The results clearly demonstrated that, by using a cut-off of $0.97\mu\text{g/L}$, S100B was able to identify those that endured “Very Severe” post-traumatic amnesia with comparable efficacy to that of the GCS (AUC = .772 and .804 respectively). Added to this, results indicated that the sensitivity and specificity of S100B identifying “Very Severe” post-traumatic amnesia (.775 and .718), was arguably better balanced than that of GCS (.525 and .949). In any case, the clinical and prognostic utility in combining serum S100B levels and GCS scores in acute TBI management is clearly evident. Clinicians making an unfavourable post-traumatic amnesia prognosis for patients with a serum S100B level greater than $0.97\mu\text{g/L}$ and a GCS score lower than 13 can do so with confidence informed by concise convergent validation.

Chapter 7

Study Two: Serum S100B and Cognitive Impairment (T₆₀)

7.1 *Aims and Hypotheses*

The focus of Study Two: T₆₀ was on predicting the cognitive impairment experienced by TBI patients 60 days following injury, based on information available at their acute presentation. Within this prognostic design, Study Two: T₆₀ aimed to quantify the accuracy that S100B holds in predicting the magnitude of ipsative impairment experienced by TBI patients, across a variety of gold-standard measures of cognitive ability. Operationally, ipsative impairment was defined in this study as being the magnitude of discrepancy between an individual's cognitive performance and the quantified estimation of their premorbid ability. This model is markedly different to normative impairment whereby impairment is defined as discrepancy from a population norm.

As discussed in the preceding literature review, higher levels of S100B have been shown to be associated with normative deficits in cognitive functioning within two weeks of emerging from post-traumatic amnesia (Watt et al., 2006), and at three and six months following injury (Ingebrigsten et al., 1999; Hermann et al., 2001). However these results have been contested by the results of other studies (Waterloo et al., 1997; De Boussard et al., 2005). Based on the associate literature, the hypotheses for Study Two: T₆₀ were:

- H₁: S100B levels would be significantly positively correlated with magnitude of ipsative impairment on measures of cognitive ability
- H₂: As per H₁, the relationship between S100B and cognitive ability would be such that ipsative impairment can be algorithmically predicted by S100B.

H₃: The accuracy of such algorithms could be improved by incorporating GCS and WPTA scores in order to control for the unique variance accounted for by these measures.

7.2 Method

7.2.1 Participants

In this study, participants who had a blood sample taken immediately following their injury, received a GCS score on admission, and completed the cognitive assessment battery 60 days post injury were included in analysis at the T₆₀ time point. The investigator conducting the cognitive assessment was blinded to the results of S100B analyses, and also to the patients' GCS and WPTA scores.

From the initial participant pool of 127 TBI patients, 53 participants met the inclusion criteria for the T₆₀ time point. However, three of the participants were suspected of not giving genuine effort during testing (by testing positive on the Dot Counting Test, described in the *Materials* section), and as such, were removed from any further analyses. 38 of the 50 T₆₀ participants received WPTA testing during their admission. Table 7.1 displays the TBI severity categorisation frequencies on the GCS and WPTA for the T₆₀ participants.

Table 7.1
Severity Categorisation Frequencies for Glasgow Coma Scale and Westmead Post-Traumatic Amnesia Scale

Glasgow Coma Scale <i>n</i> = 50	Definition	Frequency
Mild	GCS = 13<15	39 (78.00%)
Moderate	GCS = 9<12	3 (6.00%)
Severe	GCS = 3<8	8 (16.00%)
Westmead Post-Traumatic Amnesia Scale <i>n</i> = 38	Definition	Frequency
Severe	WPTA = 1 to 7 days	15 (39.47%)
Very Severe	WPTA = 8 days +	23 (60.53%)

Note. *N* = 50.

7.2.2 *Materials*

7.2.2.1 *Test of Premorbid Functioning (TOPF)*

The TOPF (Pearson, 2009) is a cognitive test that is used to make predictions of a patient's premorbid intellectual functioning. The TOPF is a recent revision of the Wechsler Test of Adult Reading (WTAR; Holdnack, 2001) – however the name was changed to reduce confusion surrounding the use of “reading” and “test” in the name of the product.

The task requires the reading of written words that are comprised of atypical grapheme-to-phoneme translations (e.g., “subtle”), as this task has been proven to be less susceptible to the effects of brain injury. The irregular pronunciation minimises the assessment of the examinee's current abilities to apply standard pronunciation rules and maximizes the assessment of their previous learning of the word.

During testing, the examinee is provided with the Test of Premorbid Functioning Word Card (Appendix D) and instructed to read each word aloud. The examiner marks the examinee's responses as correct or incorrect, and the test is discontinued following five incorrect responses. The examinees raw score is then recorded as the total number of correct responses.

The TOPF allows for “complex” and “simple” predictions of premorbid functioning. In the “complex” method, the examinee's raw score is incorporated into a regression equation that is mediated by their geographical location, years of education, and highest level of employment. In the “simple” method, the examinee's raw score alone is converted to an age-adjusted Standard Score. The present study utilised the “simple” method, as to date, the mediators of the “complex” method have not been validated with an Australian demographic sample.

The TOPF standard score has a mean of 100 and a standard deviation of 15, and this standard score is the examinee's predicted premorbid full scale IQ (FSIQ).

7.2.2.2 *The Dot Counting Test (DCT)*

The DCT (Boone, Lu, & Herzberg, 2002) is a widely utilised test used to confirm genuine task-taking effort during cognitive evaluations, rather than suppressed effort or “malingered.” This test was incorporated in the current study to identify any participants suspected of suppressing effort during evaluation – and to remove their data from statistical analyses.

The DCT task simply requires the examinee to correctly count the amount of dots presented on a page, over a series of pages with varying (i.e., non-hierarchical) difficulty. Once the examinee has completed the series of pages, an “*e*-score” is determined based on the examinee's completion time, errors made, and deviations from hierarchical performance. This study adopted the *e*-score cut-off for TBI populations, which has been validated by the authors as holding 81.6% sensitivity and 90.0% specificity for identifying “suspect effort.”

7.2.2.3 *Wechsler Adult Intelligence Scale – 4th Ed. Digit Span Subtest (WAIS-IV Digit Span)*

The WAIS-IV (Wechsler, 2008) Digit Span subtest is the most recent revision of the long used digit span task. Historically, the digit span task has involved the examinee repeating verbally presented number strings in increasing length (Digit Span Forwards), and likewise, recalling verbally presented number strings in reverse order to their presentation (Digit Span Backwards).

Unlike previous incarnations, however, the WAIS-IV Digit Span has incorporated a third presentation – namely, Digit Span Sequencing. In Digit Span Sequencing, examinees are required to recall the presented numbers in ascending order (from smallest to largest). This third presentation of the task has been suggested to increase the role of mental manipulation and places greater demand on working memory relative to the other two tasks of WAIS-IV Digit Span (Drozdzick, Wahlstrom, Zhu, & Weiss, 2012).

7.2.2.4 Wechsler Adult Intelligence Scale – 4th Ed. Figure Weights Subtest (WAIS-IV Figure Weights)

The WAIS-IV Figure Weights Subtest is a recent addition to the WAIS-IV battery, and holds the strongest standalone psychometric properties as a measure of fluid intelligence (often referred to as *Gf*) in the WAIS-IV (Lichtenberger & Kaufman, 2013), and places a high demand on quantitative reasoning. As such, McCrea and Robinson (2011) recently reviewed the subtest as being a “significant and unique innovation with few if any parallels in the prior psychometric literature.”

In the task, the examinee views scales with missing weights and selects a response from five presented options that is best suited to keep the scales balanced. The subtest is similar to previously developed “balance beam” tasks where the examinee would be required to integrate weight data with proportional distance from the fulcrum. WAIS-IV Figure Weights, however, only uses different colours and shapes as weights without the necessity of incorporating proportional distance from the fulcrum.

7.2.2.5 *Wechsler Adult Intelligence Scale – 4th Ed. Cancellation Subtest (WAIS-IV Cancellation)*

The WAIS-IV Cancellation subtest is a task that requires the examinee to scan a structured arrangement of coloured shapes and mark the targets and avoid the distracters whereby the distractors are paralleled with the targets by either shape or colour. As such, the examinee is required to discriminate and identify the targets (e.g., red squares and yellow triangles) from descriptively similar distractors (e.g., yellow squares and red triangles).

WAIS-IV Cancellation is a recent addition to the WAIS-IV battery, and supplemental to the WAIS-IV Processing Speed Index. Although the subtest is primarily considered to be a measure of processing speed and visuomotor ability, it has also been shown to be a measure of visual selective attention and dual-tasking/inhibition (McCrea & Robinson, 2011). In each of the three WAIS-IV subtests in this study (Digit Span, Figure Weights, and Cancellation), the examinee's raw score is converted to an age-adjusted scaled score, with a mean of 10 and a standard deviation of 3.

7.2.2.6 *Delis-Kaplan Executive Function Scale Verbal Fluency Test Condition 1:*

Letter Fluency (D-KEFS VFT: 1)

The D-KEFS (Delis, Kaplan, & Kramer, 2001). VFT: 1 subtest is a task that requires the examinee to simply list as many words as they can within 60 seconds that begin with a given letter, excluding proper nouns, numbers, and the same word with a different suffix. This process is repeated across three trials and the examinee's raw score (total words across all trials) is converted to an age-adjusted scaled score, with a mean of 10 and a standard deviation of 3.

Verbal fluency is a longstanding paradigm in neuropsychological assessment, and found to be highly sensitive to the cognitive deficits following TBI. The letter (or phonemic) fluency subtest has been shown to measure auditory attention, lexical retrieval, processing speed, and the more global cognitive domain of executive functioning.

7.2.2.7 Delis-Kaplan Executive Function Scale Trail Making Test Condition 4:

Number-Letter Switching (D-KEFS TMT: 4)

The D-KEFS TMT: 4 subtest is a recent rendition of the classic trail making test that has been in the public domain and use since the mid-1940s whereby the examinee is required to draw lines connecting randomly arranged numbers and letters, switching back and forth between numbers and letters in ascending order (i.e., 1, A, 2, B, 3, C etc.). However, the D-KEFS TMT: 4 revision of the classic test addresses the longstanding shortcoming in which there was a lack of sensitivity to mild executive deficits in individuals with high premorbid intellectual abilities by increasing the stimulus size (to A3, portrait orientation) and clustering “capture stimuli” (e.g., 8 close to 9, L close to M) in order to make the subtest more subtle to impairments in set-shifting.

Beyond being a test of visual scanning and motor speed, the D-KEFS TMT: 4 subtest measures executive functioning domains of cognitive flexibility, dual tasking, sequencing, and divided attention. In each of the D-KEFS subtests in this study (VFT: 1, and TMT: 4), the examinee’s raw score is converted to an age-adjusted scaled score, with a mean of 10 and a standard deviation of 3.

*7.2.2.8 BIRT Memory and Information Processing Battery Processing Speed Task
(BMIPB Speed of Information Processing).*

The BMIPB (Coughlan, Oddy, & Crawford, 2007) Speed of Information Processing subtest requires the examinee to visually scan strings of five double-digit numbers, and mark the second highest number in each string. The sum of targets that the examinee successfully completes in four minutes is recorded as their Total Score. The examinee then completes a simple motor speed task, with no additional cognitive demands to provide a Motor Speed Score.

To control for any motor deficits that may contribute to impaired processing speed, the Total score and the Motor Speed score are then cross-referenced to create an Adjusted Score. The examinee's Total Score, Motor Speed Score, and Adjusted Score are then converted to age-adjusted scaled scores, with means of 10 and standard deviations of 3.

7.2.2.9 T₀ Materials used in Study Two: T₆₀

In addition to the cognitive measures outlined above, Study Two: T₆₀ also incorporated serum S100 calcium binding protein B (S100B), the Glasgow Coma Scale (GCS), and the Westmead Post-Traumatic Amnesia Scale (WPTA). Detailed descriptions of these materials can be located in the *Materials* section for Study One: T₀.

7.2.3 Procedure

Participants were contacted by telephone and invited to participate in a cognitive evaluation (see *Materials* section) 60 days following their injury. Evaluations were conducted in an examination room and lasted approximately 40 minutes.

7.2.4 Design and Analyses for Study Two: T_{60}

In study two, patients' S100B values, GCS scores, and WPTA scores constituted the independent variables, and the magnitude of ipsative impairment on each cognitive subtest at 60 days post injury constituted the dependent variables.

For this study, ipsative impairment was defined and quantified as being the discrepancy between the participants' performance on the given cognitive subtest (Scaled Score; $M = 10$, $SD = 3$) and their expected performance given their TOPF performance (Standard Score; $M = 100$, $SD = 15$). The curve of standardised distribution with Standard Scores and Scaled Scores is displayed in Figure 7.1.

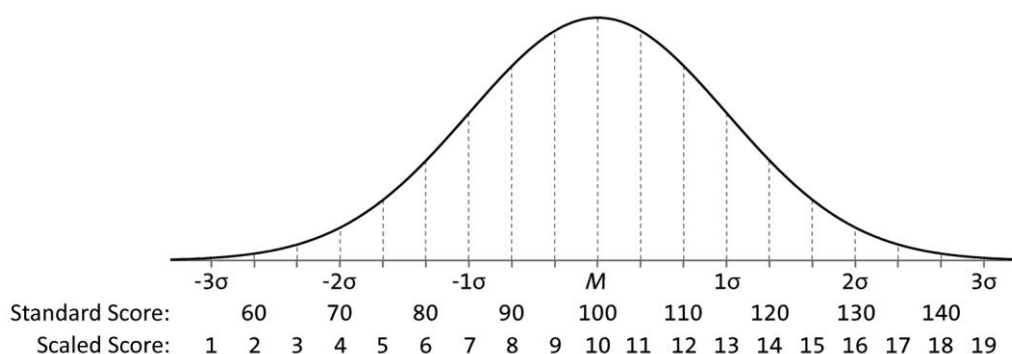


Figure 7.1. The standard distribution curve with standard scores and scaled scores

To facilitate the discrepancy analyses described above, conversions were made to cognitive subtest Scaled Scores to transform them to the Standard Score rubric. The algorithm used to convert Scaled Scores to the Standard Score rubric is depicted below in *Equation 7.1*.

$$\text{Standard Score} = (\text{Scaled Score} - 10) \times 5 + 100 \quad (\text{Eq. 7.1})$$

Two-tailed bivariate correlations with Pearson coefficients were conducted to explore statistical relationships between S100B and the existing diagnostic variables (GCS and WPTA), with impairment on each of the cognitive subtests. The ability for S100B to predict impairment was examined using robust linear regression, and subsequent regressions investigated whether the proportion of variance accounted for could be improved by including GCS scores and WPTA in each prognostic model.

7.2.5 *Statistical Software and Manipulations*

As per Study One, correlations, robust regression analyses, and receiver operating characteristics were conducted using SPSS[®] Version 21 (IBM Corp., 2012). Jarque-Bera tests for normality, Breusch-Pagan tests for heteroscedasticity, and Diagnostic and Agreement statistics were computed using Excel 2010 (Microsoft, 2010). Further information for these analyses can be found in the *Materials* section for Study One.

7.3 Results

7.3.1 Ipsative Deficit Descriptives

The mean level of premorbid functioning in the participant pool, as assessed by the TOPF was 94.12 ($SD=13.35$). Ipsative deficit for each subtest was determined by subtracting participants' actual standard score performance from their expected premorbid performance as assessed by the TOPF. Table 7.2 displays the participants' mean magnitude of ipsative deficit (in standard score points) for each of the cognitive subtests.

Table 7.2
Level of Ipsative Deficit on each of the Cognitive Subtests

Subtest	<i>M</i>	<i>SD</i>
D-KEFS Verbal Fluency	5.27	15.10
D-KEFS Trail Making	5.02	14.73
BMIPB Speed of Info Processing (Adjusted)	2.40	16.87
- <i>Total Score</i>	2.92	16.49
- <i>Motor Speed</i>	9.27	17.72
WAIS-IV Cancellation	1.71	14.06
WAIS-IV Digit Span (Total)	1.38	14.22
- <i>Forwards</i>	1.08	15.27
- <i>Backwards</i>	0.38	14.51
- <i>Sequencing</i>	4.48	16.91
WAIS-IV Figure Weights	3.30	19.21

Note. $N = 50$.

7.3.2 Correlations with Cognitive Impairment

Two-tailed Pearson's correlations were conducted between serum S100B levels, depth of coma (GCS), duration of post-traumatic amnesia (WPTA), and level of impairment on each of the cognitive subtests.

As displayed in Table 7.3, S100B was not significantly correlated with any cognitive subtest deficit; GCS was significantly correlated with the motor speed component of the BMIPB Speed of Information Processing subtest; and WPTA was significantly moderately correlated with the WAIS-IV Cancellation subtest.

Table 7.3

Correlations between S100B, GCS, and WPTA with Ipsative Deficit on each of the Cognitive Subtests

Subtest	S100B (n=50)	GCS (n=50)	WPTA (n=38)
D-KEFS Verbal Fluency	.066	.142	-.049
D-KEFS Trail Making	.008	.205	-.205
BMIPB Speed of Info Processing (Adjusted)	.196	-.034	-.246
- <i>Total Score</i>	.216	-.078	-.218
- <i>Motor Speed</i>	.173	-.371*	-.107
WAIS-IV Cancellation	.081	.053	-.391*
WAIS-IV Digit Span	.004	.130	-.074
- <i>Forwards</i>	.091	.116	-.091
- <i>Backwards</i>	.009	.015	-.001
- <i>Sequencing</i>	.005	.166	-.146
WAIS-IV Figure Weights	.234	-.217	-.042

Note. * $p < .05$

7.3.3 Predicting Cognitive Impairment

Robust regression analyses were conducted for S100B, GCS, and WPTA predicting impairment for each of the cognitive subtests. Table 7.4 below displays the R^2 values for each analysis, and the beta coefficient for each predictor. As displayed in Table 7.4, cognitive impairment could only be significantly predicted for three subtests, namely: BMIPB Speed of Information Processing (Adjusted Score); BMIPB Speed of Information Processing Total Score; and, WAIS-IV Figure Weights. With reference to beta weight coefficients, the only significant contributions made by independent variables to models were WPTA to WAIS-IV Cancellation, and GCS to WAIS-IV Figure Weights. Serum S100B made no significant contributions to any cognitive impairment models.

Table 7.4
Regression Analyses for predicting Ipsative Deficit on each of the Cognitive Subtests

Subtest	R^2	β Coefficient				p
		S100B	GCS	WPTA	Constant	
D-KEFS Verbal Fluency Test: 1	.007	.273	.150	-.051	-5.515	.949
D-KEFS Trail Making Test: 4	.043	.086	.092	-.283	-1.753	.596
BMIPB Speed of Info Processing	.110	.724	-.560	-.554	11.557	.026*
- Total Score	.105	.781	-.596	-.511	10.823	.015*
- Motor Speed	.151	.439	-.514	-1.789	19.209	.097
WAIS-IV Cancellation	.200	.237	-.818	-.700*	15.990	.090
WAIS-IV Digit Span	.009	.049	.304	-.061	-.838	.926
- Forwards	.029	.644	.665	-.065	-7.765	.734
- Backwards	.004	-.091	-.330	-.091	5.397	.976
- Sequencing	.032	-.107	.486	-.157	3.612	.621
WAIS-IV Figure Weights	.121	.619	-1.670*	-.362	22.747*	.002*

Note. $N = 38$; $p < .05$

7.3.4 Sensitivity and Specificity of Predicting “Diffuse Cognitive Impairment”

Of the 50 T₆₀ participants who sustained a TBI, 8 participants were classified as exhibiting “Diffuse Cognitive Impairment,” operationally defined as demonstrating impairment greater than one standard deviation (15 standard score points) from premorbid functioning, in at least six assessed cognitive subtests.

Receiver operating characteristics conducted on S100B and “Diffuse Cognitive Impairment” indicated an AUC of .585. As per Study One, the S100B cut off point was set at 0.97 μ g/L for these analyses. Cohen’s Kappa analysis of this cut off point was non-significant, and indicated only a slight agreement for classification, $\kappa = .095$, $p = .370$. The participants classified as exhibiting “Diffuse Cognitive Impairment” did not have significantly higher S100B levels, $t(40.003) = .840$, $p = .261$

Receiver operating characteristics conducted on GCS and “Diffuse Cognitive Impairment” indicated an AUC of .579. Consistent with Study One, a GCS score of 12 (“Moderate” severity) was used as the cut off point for these analyses. Cohen’s Kappa analysis of this cut off point was also non-significant, and indicated only a

below chance agreement for classification, $\kappa = -.114$, $p = .406$. The participants classified as exhibiting “Diffuse Cognitive Impairment” did not have significantly lower GCS scores, $t(14.110) = -1.107$, $p = .144$

Receiver operating characteristics conducted on WPTA and “Diffuse Cognitive Impairment” indicated an AUC of .721. As per Study One, the WPTA “Very Severe” classification (≥ 8 days) was used as the cut off point for these analyses. Cohen’s Kappa analysis of this cut off point was also non-significant, and indicated only a slight agreement for classification, $\kappa = .050$, $p = .635$. The participants classified as exhibiting “Diffuse Cognitive Impairment” did not have significantly higher WPTA scores, $t(6.374) = 1.676$, $p = .503$.

The Receiver Operator Characteristics for S100B (AUC = .585), GCS (AUC = .579) and WPTA (AUC = .721) in identifying “Diffuse Cognitive Impairment” are depicted below in Figure 7.2.

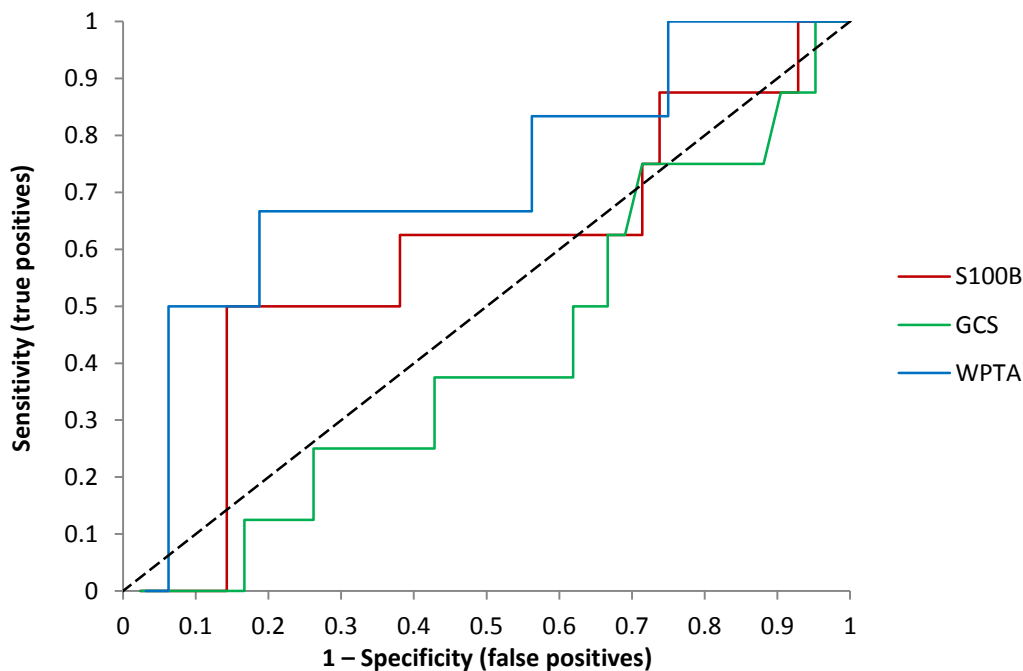


Figure 7.2. Receiver Operating Characteristics of S100B, GCS and WPTA with “Diffuse Cognitive Impairment.”

The diagnostic and agreement statistics for S100B using $0.97\mu\text{g/L}$; GCS using ≥ 12 ; and, WPTA using ≥ 8 days as respective cut offs for identifying “Diffuse Cognitive Impairment” are displayed below in Table 7.5.

Table 7.5

Diagnostic and Agreement Statistics for identifying “Diffuse Cognitive Impairment” using S100B, GCS, and WPTA

	S100B		GCS		WPTA	
Sensitivity	.625	(.245 – .915)	.125	(.003 – .527)	.667	(.223 – .957)
Specificity	.548	(.387 – .702)	.738	(.580 – .861)	.438	(.264 – .623)
Efficiency	.560	(.413 – .700)	.640	(.492 – .771)	.474	(.310 – .642)
False Positive Rate	.452	(.298 – .613)	.262	(.139 – .420)	.563	(.377 – .736)
False Negative Rate	.375	(.085 – .755)	.875	(.473 – .997)	.333	(.043 – .777)
Misclassification Rate	.440	(.300 – .345)	.360	(.229 – .458)	.526	(.358 – .403)

Note. 95% confidence intervals are displayed in parentheses. S100B cut off $\geq 97\mu\text{g/L}$, $n = 50$; GCS cut off ≤ 12 , $n = 50$; WPTA cut off ≥ 8 days, $n = 38$.

Figures 7.3 through 7.8 on the following pages illustrate the respective sensitivity and specificity of S100B, GCS and WPTA in identifying “Diffuse Cognitive Impairment” (ipsatively impaired on ≥ 6 subtests). The respective cut-offs for each independent variable (established above) are depicted in each plot.

Figures 7.3 and 7.4 below plot the sensitivity and specificity of S100B in identifying “Diffuse Cognitive Impairment,” the cut off level of $.97\mu\text{g/L}$ is depicted in the plot.

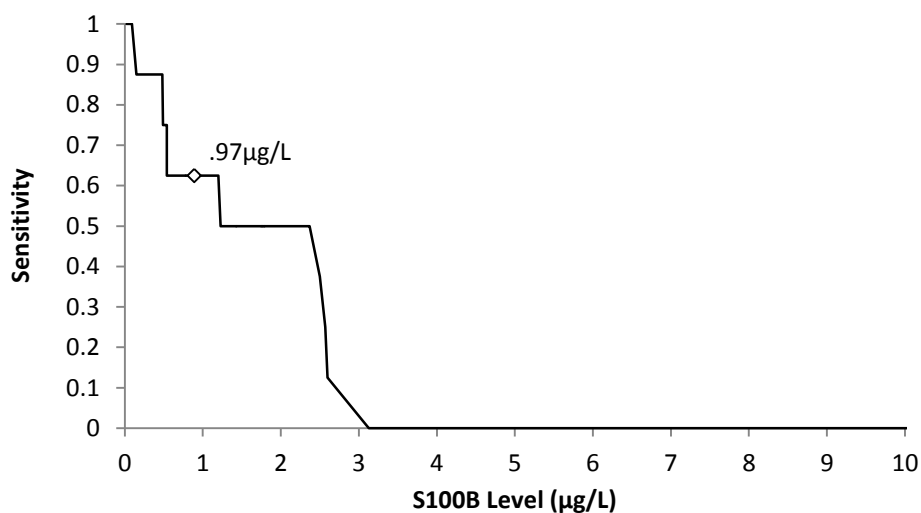


Figure 7.3. The sensitivity of serum S100B levels in identifying “Diffuse Cognitive Impairment.”

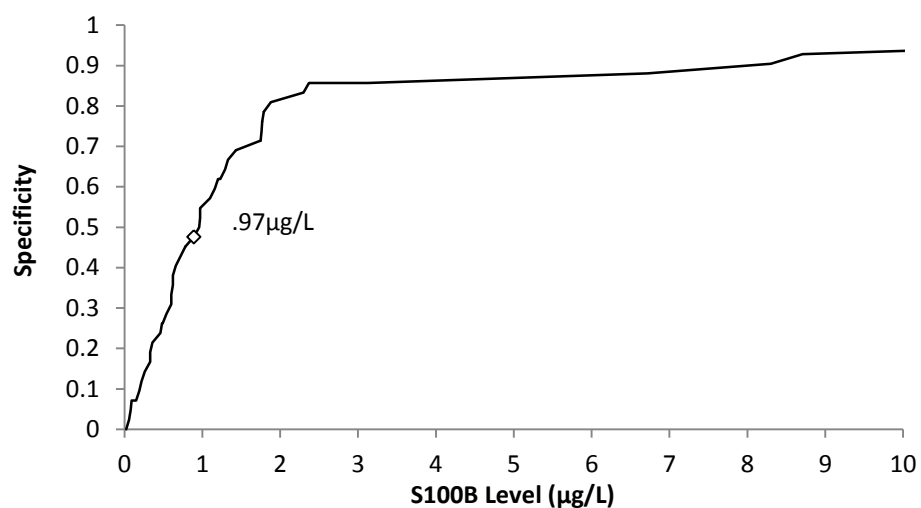


Figure 7.4. The specificity of serum S100B levels in identifying “Diffuse Cognitive Impairment.”

Figures 7.5 and 7.6 below plot the sensitivity and specificity of depth of coma (as measured by the GCS) in identifying “Diffuse Cognitive Impairment,” the GCS cut off score of 12 is depicted in the plot.

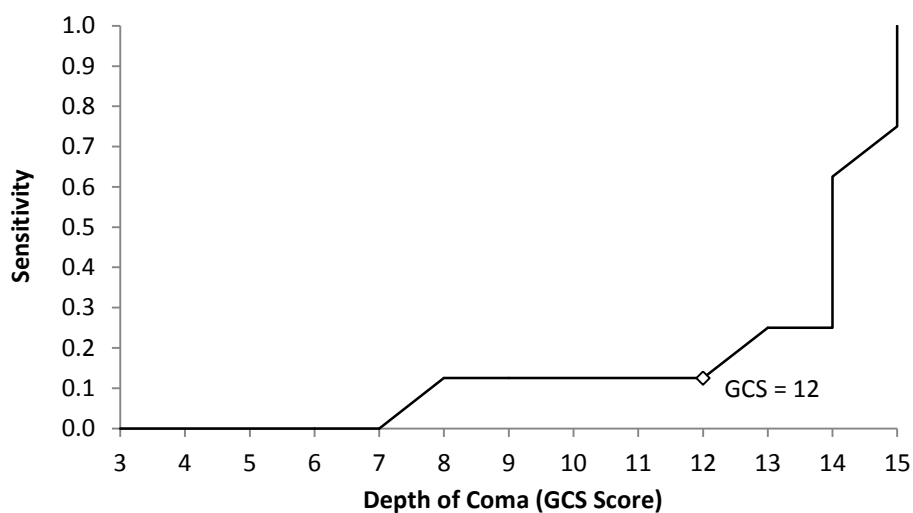


Figure 7.5. The sensitivity of GCS scores in identifying “Diffuse Cognitive Impairment.”

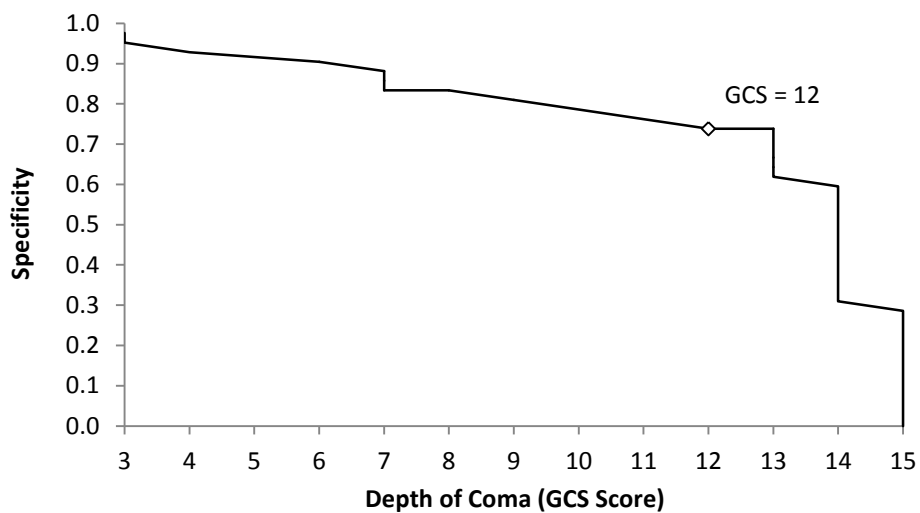


Figure 7.6. The specificity of GCS scores in identifying “Diffuse Cognitive Impairment.”

Figures 7.7 and 7.8 below plot the sensitivity and specificity of duration of post-traumatic amnesia (as measured by the WPTA) in identifying “Diffuse Cognitive Impairment,” the WPTA cut off level of ≥ 8 days is depicted in the plot.

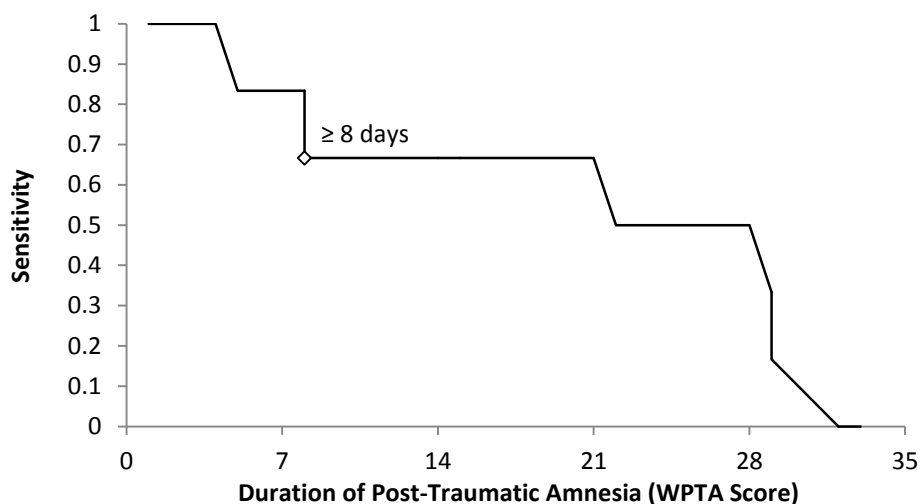


Figure 7.7. The sensitivity of WPTA Scores in identifying “Diffuse Cognitive Impairment.”

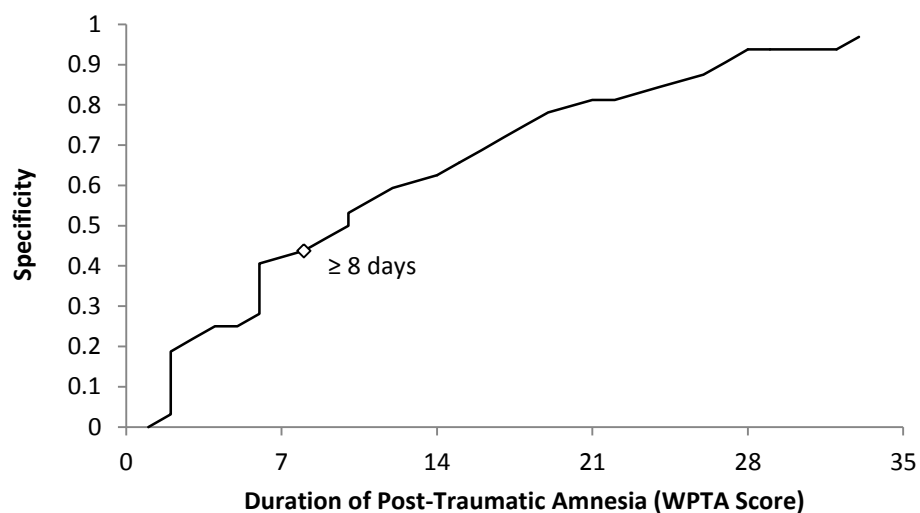


Figure 7.8. The specificity of WPTA scores in identifying “Diffuse Cognitive Impairment.”

7.4 Discussion

As an ideal, a proteomic biomarker of TBI outcome should indicate damage to neurological networks and also to specific brain cells that are known to support cognitive functioning. As such, it would be beneficial for the prognostication of cognitive impairment following TBI to explore the mechanisms that underlie changes in proposed biomarkers of TBI (Papa et al., 2013). In alignment with the phases of biomarker development modelled by Pepe et al. (2013), the present study investigated the prognostic utility of serum S100B with reference to cognitive impairment experienced by TBI patients 60 days post injury. More specifically, the present study quantified the accuracy that S100B holds in predicting the magnitude of ipsative impairment experienced by TBI patients, across a variety of standardised measures of cognitive ability. This study utilised an ipsative impairment model, rather than a normative deficit model, in order to investigate discrepancies between participants' cognitive performances and quantifiable estimations of their premorbid abilities.

The associated research, discussed in the preceding literature review, indicates that disrupted S100B levels are associated with altered learning and memory in rodent models of injury (Mello e Souza et al., 2000; Winocur et al., 2001). Further, within human models of injury, higher levels of S100B have been shown to be associated with normative deficits in cognitive functioning within two weeks of emerging from post-traumatic amnesia (Watt et al., 2006), and at three and six months following injury (Ingebrigsten et al., 1999; Hermann et al., 2001). As such, a meta-analysis conducted by Sun and Feng (2013) concluded that a substantial body of evidence now exists to suggest that a relationship exists between post-traumatic biomarker dysfunction and cognitive impairment.

In alignment with the aforementioned relationship, this study hypothesised that serum S100B levels would be significantly correlated with the magnitude of ipsative impairment on each of the measures of cognitive ability. It was further hypothesised that ipsative impairment in each domain could be algorithmically predicted by serum concentrations of S100B. Contrary to these hypotheses, however, S100B was not significantly correlated with ipsative impairment on any of the cognitive subtests utilised in this study. Further, regression analyses for cognitive impairment illustrated that S100B made no significant contributions to any prognostic models. Despite the absence of clinical significance, all correlations between serum concentrations of S100B and ipsative cognitive impairment were found to be in the hypothesised direction. This direction-consistent result was also found for duration of post-traumatic amnesia, but not found for depth of coma.

7.4.1 Comparisons to the Empirical Literature

The current findings are in contrast to some of the results contained in the pioneering study conducted by Waterloo et al. (1997). The researchers compared cognitive abilities, one year post injury, between seven participants with mild TBI and seven age- and sex-matched controls without detectable levels of S100B. Elevated levels of S100B were found to be correlated with cognitive impairment on measures of complex reaction time which demanded an increased amount of attention and information processing. No significant relationships were found, however, between concentrations of S100B and measures of immediate memory, delayed memory, dual-tasking, or perseveration/inhibition. Of note, the published data suggests that the researchers used the raw scores of subtests as the dependent variables in their experimental design rather than scaled scores for normative

comparison. Further, Waterloo et al.'s findings are limited to mild TBIs, and the sample size of seven limits the strength of the conclusions and implications that are able to be drawn from the study for cognitive outcomes following TBI.

The present study's findings also conflict with those of Ingebrigsten et al. (1999)'s study of 50 mild TBI patients' cognitive outcomes three months post injury. The researchers reported trends towards significance for higher serum S100B levels being associated with impaired attention, memory, and speed of information processing. Given the absence of statistical significance and reliance on trends to declare implications, it could be argued that these conclusions are somewhat bold. Further, it should be noted that, methodologically, Ingebrigsten et al. recruited only patients with mild injuries, and stratified their participants by the presence or absence of S100B.

Similar to Waterloo et al. (1997) and Ingebrigsten et al. (1999), Herrmann et al. (2001) found that patients with higher serum concentrations of S100B exhibited more pronounced cognitive impairment at six months post injury. In their study of 29 mild TBI patients, the researchers reported correlations between S100B levels and poorer performance on measures of attention, perceptual reasoning, and verbal fluency. It is important to note, however, that the persistence of cognitive dysfunction in 69% of their participant sample is remarkably atypical with mild TBI populations. Further, the participants' premorbid intellectual functioning was not incorporated into the experimental design – consequently, it is possible that the participants' poor performances may have reflected lower premorbid levels of cognitive functioning and other nonorganic factors that influence injury. The present study made attempts to control for these factors by assessing premorbid functioning, age-referencing performances, and screening for less than genuine effort.

Perhaps the study of most direct comparison to the present study is that of Watt et al. (2006). Using a matched-control longitudinal design, the researchers investigated the neuropsychological performance of 23 severe TBI patients within two weeks of emerging from post-traumatic amnesia, as assessed by the WPTA. Results indicated that acute S100B samples were correlated with performance on tasks involving visual memory, verbal memory and list learning, auditory working memory, speed of information processing, verbal fluency, and reaction time. However, no significant correlations were found between S100B concentrations and Full Scale IQ, Verbal IQ, Performance IQ, attention/concentration, or delayed recall.

Despite similar experimental designs, it should be noted that the current study did not incorporate any measures of immediate or delayed memory or reaction time. Further, participants in Watt et al's study underwent neuropsychological assessment in the sub-acute setting (less than one month post injury) where deficits are most pronounced, whereas the present study conducted assessments in the post-acute/recovery stage (greater than two months post injury) where individual deficits are most variable.

7.4.2 *Conclusions*

While results indicated that S100B concentrations were not associated with the magnitude of any ipsative cognitive impairment, it should be acknowledged that GCS and WPTA scores could not prognosticate impairment either. None of the independent variables held significant cut-offs for correctly classifying "diffuse cognitive impairment" as operationalised in this design. While the WPTA held a fair area under the curve for classification, the participants classified as exhibiting "diffuse cognitive impairment" did not experience significantly longer periods in

post-traumatic amnesia. In summary, the results from this study illustrate that although post-traumatic amnesia is known to be the best indicator of functional deficits that follow TBI (Khan et al., 2003), enduring future cognitive impairment is remarkably difficult to predict from information available in the acute setting.

More broadly, this study extends the associated biomarker literature by incorporating an ipsative impairment model of post injury cognitive functioning as a dependent variable. Beyond S100B specifically, there is potential for biomarkers being used as a screening tool for injury at a cellular level – and in doing so, clinicians may be able to intervene pharmacologically in efforts to interrupt the development of secondary pathologies known to affect cognitive functioning (Sun & Feng, 2013). As such, any proposed biomarker undergoing clinical validation for TBI management would not only benefit from incorporating functional longitudinal design, but also from developing the understanding of the structural and cellular mechanisms that underlie changes in the biomarker itself.

From a translational perspective, this study illustrates that any accurate prognoses relating to cognitive impairment following TBI require acute neuropsychological assessment and consultation, and that prognoses based on biological factors alone while in an acute setting remains elusive, if not illusive. For the patient who has suffered a TBI, clinical examination of indices of brain injury severity, medical imaging, comprehensive history taking and neuropsychological assessment remains the best practice for attaining an accurate prognosis. That being said, as new biomarkers emerge, the onus is on the medical and neuropsychological literature to incorporate these biomarkers into experimental design – and potentially, into clinical practice.

Chapter 8

Study Three: Serum S100B and Post-Concussion Syndrome and Quality of Life (T₁₈₀)

8.1 *Aims and Hypotheses*

The focus of Study Three: T₁₈₀ was on predicting the severity of symptoms of post-concussion syndrome and quality of life experienced by TBI patients 180 days following injury, based on information available at their acute presentation. As such, Study Three: T₁₈₀ aimed to quantify the accuracy that S100B holds in predicting the presence and severity of PCS symptoms and quality of life, and whether the accuracy of such predictions can be improved by incorporating GCS and WPTA scores. In short, the aim was to quantify whether the symptoms that TBI patients experience six months post injury can be predicted from their acute presentation.

As discussed in the preceding literature review, S100B has been shown to be associated with symptoms of PCS at two weeks post-injury (Savola & Hillborn, 2003), and three months post-injury (Bazarian et al., 2006). Further, S100B has been shown to be associated with poorer quality of life (Woertgen et al., 2002), prolonged time to return to work (Stranjalis et al., 2004; Metting et al., 2012), poorer somatic health (Stålnacke et al., 2013), and symptoms related to stress disorders (Sojka et al., 2006). Based on the literature, the hypotheses for Study Three: T₁₈₀ were:

- H₁: S100B levels would be significantly positively correlated with severity of symptoms of post-concussion syndrome.
- H₂: S100B levels would be significantly negatively correlated with domains associated with quality of life.

H₃: The sensitivity and specificity of S100B in identifying post-concussion syndrome severity and poor quality of life would be superior to that of GCS and WPTA.

8.2 Method

8.2.1 Participants

Participants who had blood taken immediately following their injury, received a GCS score on admission, and completed the British Columbia Post-Concussion Symptom Inventory and The Quality of Life Inventory (both inventories discussed below in *Materials*) 180 days post injury were included in analysis at the T₁₈₀ time point.

From the initial participant pool of 127 TBI patients, 52 participants met the inclusion criteria for the T₁₈₀ time point. One of the participants' blood samples encountered an error during analysis, and therefore was not included in statistical analyses incorporating S100B. 36 of the 52 T₁₈₀ participants received WPTA testing during their admission. Table 8.1 displays the TBI severity categorisation frequencies on the GCS and WPTA for the T₁₈₀ participants.

Table 8.1
TBI Severity Categorisation Frequencies for Glasgow Coma Scale and Westmead Post-Traumatic Amnesia Scale

Glasgow Coma Scale <i>n</i> = 52	Definition	Frequency
Mild	GCS = 13<15	44 (84.62%)
Moderate	GCS = 9<12	3 (5.77%)
Severe	GCS = 3<8	5 (9.62%)
Westmead Post-Traumatic Amnesia Scale <i>n</i> = 36	Definition	Frequency
Severe	WPTA = 1 to 7 days	18 (50%)
Very Severe	WPTA = 8 days +	18 (50%)

Note. *N* = 52.

8.2.2 *Materials*

8.2.2.1 *British Columbia Post-Concussion Symptom Inventory (BC-PSI)*

The BC-PSI (Iverson, 2006) is a 16 item self-report measure used to assess the presence and severity of post-concussion symptoms across the last two weeks. The examinee simply rates the frequency and intensity of thirteen items, and then rates the effect of three life problems on daily living. The thirteen symptoms measured by the BC-PSI are: headaches; dizziness/light-headed; nausea/feeling sick; fatigue; extra sensitive to noises; irritable; feeling sad; nervous or tense; temper problems; poor concentration; memory problems; difficulty reading; and poor sleep. The frequency of each symptom is rated on a six-point Likert scale: 0 = not at all; 1 = 1-2 times; 2 = several times; 3 = often; 4 = very often; and, 5 = constantly. Similarly, the severity of each symptom is also rated on a six point Likert scale: 0 = not at all; 1 = very mild problem; 2 = mild problem; 3 = moderate problem; 4 = severe problem; and, 5 = very severe problem.

Following the thirteen symptom items, the three life problems of daily living measured by the BC-PSI are: does alcohol affect you more than in the past?; do you find yourself worrying and dwelling on the symptoms above?; and, do you believe you have damage to your brain? The magnitude of each life problem of daily living is rated on a five point Likert scale: 1 = not at all; 3 = somewhat; 5 = very much.

For each of the thirteen BC-PSI symptoms, the frequency and the severity are multiplied to create Product Scores (min. = 0, max. =25). For clinical interpretation, Product Scores are then converted to Item Scores (0-1 = 0; 2-3 = 1; 4-6 = 2; 8-12 = 3; and, 15+ = 4) and classified on the following reference ranges: 0 = “none;” 1-2 = “mild;” 3 = “moderate;” and, 4 = “severe.” Item Scores are totalled for a BC-PSI Total Score to reflect global post-concussion symptom severity. For clinical interpretation, BC-PSI Total scores are classified based on the following reference ranges: 0 = “low;” 1-9 = “normal;” 10-14 = “unusually high;” and, 15+ = “extremely high.” Product Scores, BC-PSI Total Scores, and clinical classifications are used for this study’s analyses.

8.2.2.2 *The Quality of Life Inventory (QOLI)*

The QOLI (Frisch, 1994) is a sixteen item self-report questionnaire that is used to measure a patients overall life satisfaction. The questionnaire requires the examinee to rate their current level of importance and satisfaction across 16 domains of quality of life. The domains measured by the QOLI are: Health; Self Esteem; Goals and Values; Money; Work; Play; Learning; Creativity; Helping; Love; Friends; Children; Relatives; Home; Neighbourhood; Community

The importance of each domain is rated on a three-point unipolar Likert scale, namely 0 = not important; 1 = important; and, 2 = extremely important. Unlike the importance scale, the satisfaction of each domain is rated on a six point bipolar Likert scale, namely -3 = very dissatisfied; -2 = somewhat dissatisfied; -1 = a little dissatisfied; 1 = a little satisfied; 2 = somewhat satisfied; and 3 = very satisfied.

For each of the sixteen QOLI domains, the importance and satisfaction scores are multiplied to create Weighted Scores (min. -6, max. 6). Total Scores are then calculated by dividing the sum of Weighted Scores by the number of domains that the examinee identified as being either “important” or “extremely important” (i.e., “not important”). Total Scores are then converted to *T*-Scores, with a mean of 50 and a standard deviation of 10. For clinical interpretation, overall quality of life is classified based on the following *T*-Score reference ranges: 0-36 = “Very Low;” 37-42 = “Low;” 43-57 = “Average;” and, 58-77 = “High.”

Confirmatory factor analyses conducted by Thomas, McGrath and Skilbeck (2012), using a non-clinical Australian sample, found a three-factor structure to the QOLI. The authors classified the three factors as: “Self-Functioning & Activity” (Health, Self-Esteem, Work, and Goals and Values); “Self-Actualisation” (Play, Learning, Creativity, and Helping); and, “Family and Environment” (Money, Love, Relatives, Home, Neighbourhood, and Community).

Weighted Scores for each domain, Thomas et. al (2012) factor scores, QOLI Total Scores, *T*-Scores, and clinical classifications (based on *T*-Scores) are used for this study’s analyses.

8.2.2.3 *T₀ Materials used in Study Three: T₁₈₀*

In addition to the post-concussion syndrome and quality of life measures outlined above, Study Three also incorporated serum S100 calcium binding protein B (S100B), the Glasgow Coma Scale (GCS), and the Westmead Post-Traumatic Amnesia Scale (WPTA). These materials are outlined in the *Materials* section for Study One: *T₀*.

8.2.3 Procedure

Participants were sent a mail-out pack, 180 days following their injury. Mail-out packs consisted of a covering letter, a brief questionnaire, a QOLI form and a BC-PSI form (all contained in Appendix E), and a stamped addressed return envelope. Participants had the option to not put their name on any of the T₁₈₀ materials as each form had been pre-named with their de-identified study code.

8.2.4 Design and Analyses

In study three, patients' S100B values, GCS scores, and WPTA scores constituted the independent variables, and the severity of post-concussion symptoms (as measured by the BC-PSI) and their satisfaction with domains of quality of life (as measured by the QOLI) 180 days post-injury constituted the dependent variables.

Relationships between the independent and dependent variables were explored by conducting two-tailed bivariate correlations with Pearson coefficients. For all significant correlations between S100B and BC-PSI symptoms and QOLI domains, linear regressions were conducted, and subsequent regressions investigated whether the proportion of variance accounted for could be improved by including GCS scores and WPTA in each prognostic model. Jarque-Bera tests were conducted on the skewness and kurtosis of each model to test for normality of distribution, and Breusch-Pagan heteroscedasticity analyses were conducted on each model to investigate whether the variance (actual versus predicted) in the residuals were dependent on the value of the dependent variable (severity of the BC-PSI symptom; "Weighted Satisfaction" of the QOLI domain).

Diagnostic and agreement statistics and receiver operating characteristics were then conducted to compare the respective sensitivity, specificity, and area under the curve (AUC) of S100B, GCS, and WPTA in independently identifying future “Unusually High” and “Extremely High” post-concussion syndrome (as measured by the BC-PSI), and “Low” and “Very Low” quality of life (as measured by the QOLI).

8.2.5 *Statistical Software and Manipulations*

As per Study One, correlations, robust regression analyses, and receiver operating characteristics were conducted using SPSS® Version 21 (IBM Corp., 2012). Jarque-Bera tests for normality, Breusch-Pagan tests for heteroscedasticity, and Diagnostic and Agreement statistics were computed using Excel 2010 (Microsoft, 2010). Further information for these analyses can be found in the *Method* section for Study One.

8.3 **Post-Concussion Syndrome Results**

As per the BC-PSI protocol (Iverson, 2006), the frequency (how often) and intensity (how bad) of each BC-PSI item were multiplied to produce product scores. Product scores are used to make symptom classification as “none,” “mild,” or “moderate-severe.” Product scores were added together to create total scores. Total scores are used to make total classification as “low,” “normal,” “unusually high,” and “extremely high.” Table 8.2 displays frequencies for each BC-PSI symptom and Total BC-PSI score at each classification level.

Table 8.2
Classification Frequencies for BC-PSI Symptom Product Scores and Total Score

Symptom	None	Mild	Moderate-Severe	
Headaches	36 (69.23%)	12 (23.08%)	4 (7.69%)	
Dizziness/light-headed	34 (65.38%)	13 (25.00%)	5 (9.62%)	
Nausea/feeling sick	45 (86.54%)	5 (9.62%)	2 (3.85%)	
Fatigue	25 (48.08%)	14 (26.92%)	13 (25.00%)	
Extra sensitive to noises	30 (57.69%)	13 (25.00%)	9 (17.31%)	
Irritable	34 (65.38%)	14 (26.92%)	4 (7.69%)	
Feeling Sad	27 (51.92%)	17 (32.69%)	8 (15.38%)	
Nervous or tense	33 (63.46%)	11 (21.15%)	8 (15.38%)	
Temper problems	37 (71.15%)	10 (19.23%)	5 (9.62%)	
Poor concentration	32 (61.54%)	11 (21.15%)	9 (17.31%)	
Memory problems	28 (53.85%)	10 (19.23%)	14 (26.92%)	
Difficulty reading	36 (69.23%)	9 (17.31%)	7 (13.46%)	
Poor sleep	26 (50.00%)	11 (21.15%)	15 (28.85%)	
	Low	Normal	Unusually High	Extremely High
Total BC-PSI Score	7 (13.46%)	19 (36.54%)	10 (19.23%)	16 (30.77%)

Note. $N = 52$.

8.3.1 Correlations with Symptoms of Post-Concussion Syndrome

Two-tailed Pearson's correlations were conducted between serum S100B levels, depth of coma (GCS), duration of post-traumatic amnesia (WPTA), and symptoms of post-concussion syndrome as assessed by the BC-PSI.

As displayed in Table 8.3, S100B was significantly moderately correlated with nausea/feeling sick, feeling sad, poor concentration, and worrying and dwelling on symptoms; GCS was significantly moderately correlated with memory problems; and WPTA was significantly moderately correlated with poor concentration, memory problems, and the extent to which participants believed they have damage to their brain.

Table 8.3

Correlations between S100B, GCS, and WPTA with the Symptoms, Total, and Classification of the BC-PSI

Symptom	S100B (n=51)	GCS (n=52)	WPTA (n=36)
Headaches	-.027	-.117	.072
Dizziness/light-headed	.196	-.054	.229
Nausea/feeling sick	.310*	-.045	.273
Fatigue	.105	-.121	.182
Extra sensitive to noises	-.123	-.005	.080
Irritable	.119	-.112	.003
Feeling Sad	.337*	-.144	.253
Nervous or tense	.230	-.075	.209
Temper problems	.168	-.237	.321
Poor concentration	.342*	-.059	.337*
Memory problems	.227	-.311*	.418*
Difficulty reading	.006	.092	.128
Poor sleep	.153	.122	.067
Alcohol affecting more	.101	-.023	-.072
Worrying and dwelling on symptoms	.319*	.041	.250
Believed damage to your brain	.215	-.273	.493**
Total Score	.218	-.138	.264
Classification	.233	-.215	.298

Note. * $p < .05$ ** $p < .01$, two-tailed; $N = 52$.

8.3.2 Predicting Severity of Post-Concussion Syndrome Symptoms

Robust linear regression analyses were conducted for S100B predicting each of the significantly correlated symptoms of post concussion syndrome, and Jarque-Bera tests for normality were conducted for each regression model. Depth of coma and duration of post-traumatic amnesia were added to each model to investigate any significant contributions to variance accounted for by each model.

Breusch-Pagan heteroscedasticity analyses were then conducted for each model to investigate whether the variance (actual versus predicted) in the residuals of each model are dependent on the value of the dependent variable (severity of the symptom).

8.3.3 BC-PSI Total Score

After removal of one outlier case, serum S100B levels significantly predicted the severity of the BC-PSI Total Score (sum of the product scores for each BC-PSI symptom), $R^2 = .089$, $F(1, 49) = 4.707$, $p < .05$. The Jarque-Bera test for normality of this model was significant $\chi^2(2, N=50) = 14.138$, $p < .001$, and the algorithm for this model is presented in *Equation 8.1*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.1* $R^2 = .094$, $\Delta R^2 = .005$ $F(2, 49) = 2.434$, $p = .099$, nor did duration of post-traumatic amnesia $R^2 = .169$, $\Delta R^2 = .051$ $F(2, 33) = 3.157$, $p = .056$.

$$\text{BC-PSI Total Score} = (\text{S100B} \times 2.881) + 27.986 \quad (\text{Eq. 8.1})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.1* (S100B predicting BC-PSI Total Score) was significant, $\chi^2(1, N=50) = 8.170$, $p < .001$, and robust regression analysis revealed that S100B was no longer a significant predictor of BC-PSI Total Scores after accounting for heteroscedasticity of the model ($\beta=1.623$, $p < .05$). A post-hoc bivariate Pearson's correlation revealed a significant moderate correlation between BC-PSI Total Score, and magnitude of error in the residuals ($.782$, $p < .001$). The residual error across BC-PSI Total Scores for *Equation 8.1* can be found in Appendix F.

8.3.4 *Sensitivity and Specificity of Predicting “Unusually High” Post-Concussion Syndrome*

Of the 52 T₁₈₀ participants who sustained a TBI, 26 participants were classified in the “Unusually High” ($n = 10$) or “Extremely High” ($n = 16$) range of post-concussion syndrome severity as measured by the BC-PSI Total Score. The following diagnostic and agreement statistics are for identifying the participants who experienced at least “Unusually High” post-concussion syndrome severity ($n = 26$).

Receiver operating characteristics conducted on S100B and “Unusually High” BC-PSI Total Scores indicated an AUC of .552. As per Study One and Study Two, the S100B cut off point for identifying “Unusually High” BC-PSI Total Scores was set at 0.97µg/L for these analyses. Cohen’s Kappa analysis of this cut off point was non-significant, and indicated only a slight agreement for classification, $\kappa = .176, p = .210$. The participants classified as exhibiting “Unusually High” BC-PSI Total Scores did not have significantly higher S100B levels, $t(42.040) = -.920, p = .174$.

Receiver operating characteristics conducted on GCS and “Unusually High” BC-PSI Total Scores indicated an AUC of .408. Consistent with Study One and Study Two, a GCS score of 12 (“Moderate” severity) was used as the cut off point for identifying “Unusually High” BC-PSI Total Scores. Cohen’s Kappa analysis of this cut off point was also non-significant, and indicated only a slight agreement for classification, $\kappa = .154, p = .124$. The participants classified as exhibiting “Unusually High” BC-PSI Total Scores had significantly lower GCS scores, $t(35.934) = 1.671, p < .01$.

Receiver operating characteristics conducted on WPTA and “Unusually High” BC-PSI Total Scores indicated an AUC of .557. The WPTA “Very Severe” classification (≥ 8 days) was used as the cut off point for identifying “Unusually High” BC-PSI Total Scores. Cohen’s Kappa analysis of this cut off point was also non-significant, and indicated a fair agreement for classification, $\kappa = .111$, $p = .505$. The participants classified as exhibiting “Unusually High” BC-PSI Total Scores had significantly higher WPTA scores, $t(30.086) = -1.029$, $p < .05$.

The Receiver Operator Characteristics for S100B (AUC = .585), GCS (AUC = .579) and WPTA (AUC = .721) in identifying “Unusually High” BC-PSI scores are depicted below in Figure 8.01.

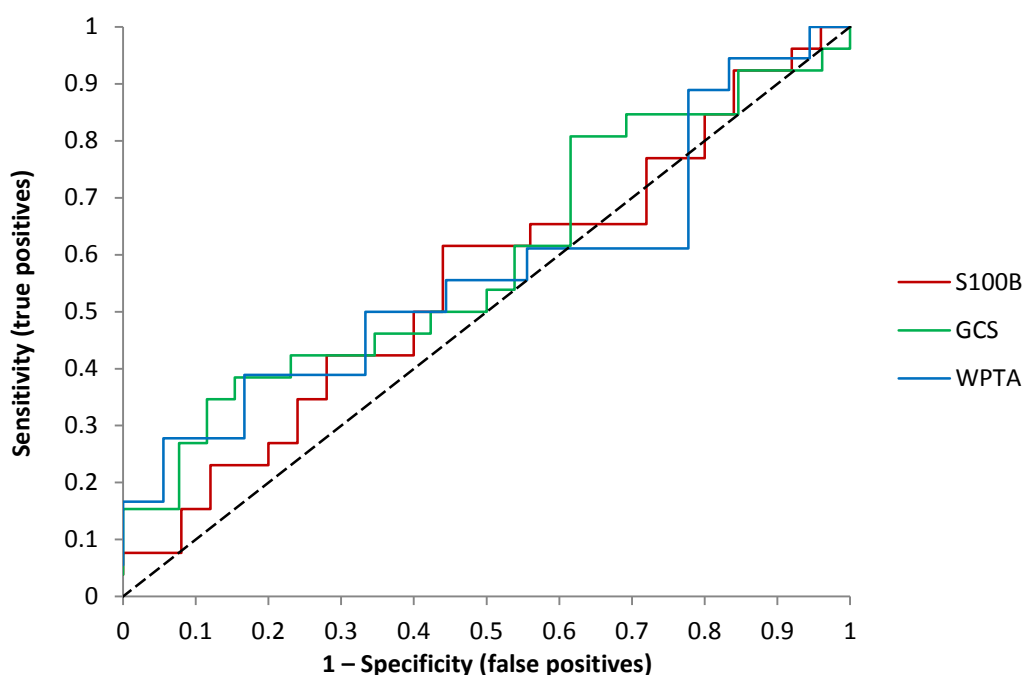


Figure 8.01. Receiver Operating Characteristics of S100B, GCS and WPTA with “Unusually High” BC-PSI Total Scores.

The diagnostic and agreement statistics for S100B using $0.97\mu\text{g/L}$; GCS using ≥ 12 ; and, WPTA using ≥ 8 days as respective cut offs for identifying “Unusually High” BC-PSI Total Scores are displayed below in Table 8.4.

Table 8.4

Diagnostic and Agreement Statistics for identifying “Unusually High” BC-PSI Scores using S100B, GCS, and WPTA

	S100B		GCS		WPTA	
Sensitivity	.615	(.406 – .798)	.231	(.090 – .436)	.556	(.308 – .785)
Specificity	.560	(.349 – .756)	.923	(.749 – .991)	.556	(.308 – .785)
Efficiency	.588	(.442 – .724)	.577	(.432 – .713)	.556	(.308 – .785)
False Positive Rate	.440	(.244 – .651)	.077	(.009 – .251)	.444	(.215 – .692)
False Negative Rate	.385	(.202 – .594)	.769	(.564 – .910)	.444	(.215 – .692)
Misclassification Rate	.412	(.276 – .548)	.423	(.287 – .689)	.444	(.279 – .619)

Note. 95% confidence intervals are displayed in parentheses. S100B cut off $\geq 97\mu\text{g/L}$, $n = 51$; GCS cut off ≤ 12 , $n = 52$; WPTA cut off ≥ 8 days, $n = 36$.

Figures 8.02 through 8.07 on the following pages illustrate the respective sensitivity and specificity of S100B, GCS and WPTA in identifying “Unusually High” post-concussion syndrome severity (BC-PSI Total Score ≥ 10). The respective cut-offs for each independent variable (established above) are depicted in each plot.

Figures 8.02 and 8.03 below plot the sensitivity and specificity of S100B in identifying “Unusually High” post-concussion syndrome severity (BC-PSI Total Score), the cut off level of $.97\mu\text{g/L}$ is depicted in the plot.

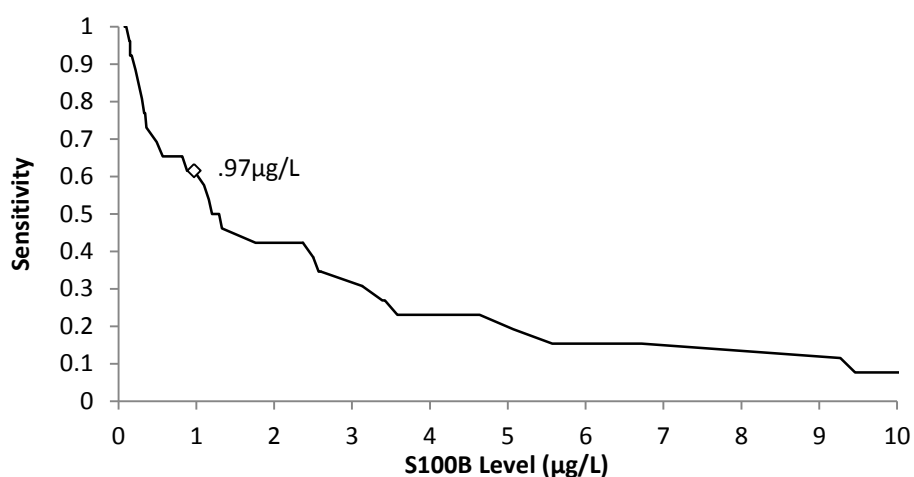


Figure 8.02. The sensitivity of serum S100B levels in identifying “Unusually High” post-concussion syndrome severity.

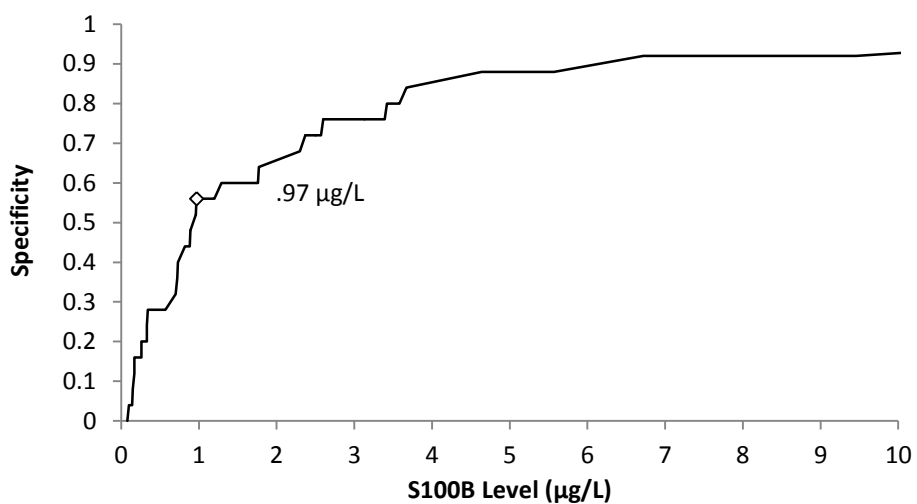


Figure 8.03. The specificity of serum S100B levels in identifying “Unusually High” post-concussion syndrome severity.

Figures 8.04 and 8.05 below plot the sensitivity and specificity of depth of coma (as measured by the GCS) in identifying “Unusually High” post-concussion syndrome severity (BC-PSI Total Score), the GCS cut off score of 12 is depicted in the plot.

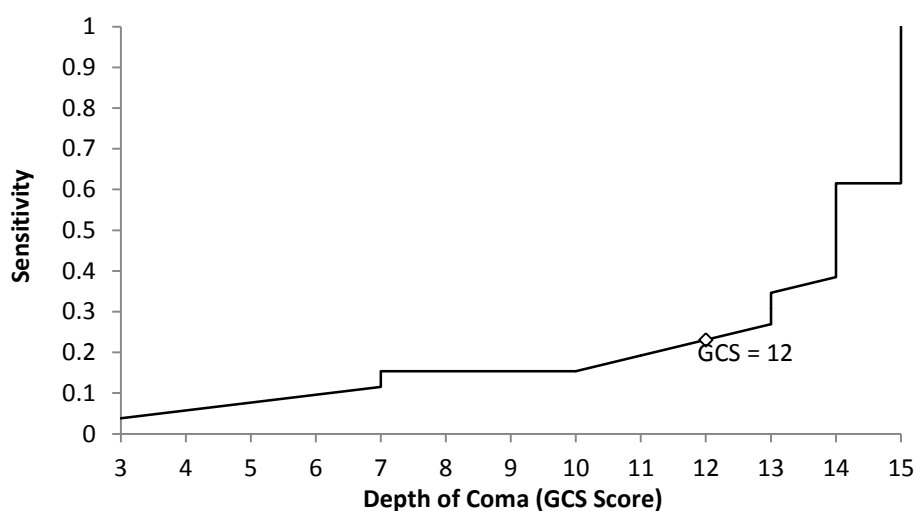


Figure 8.04. The sensitivity of GCS scores in identifying “Unusually High” post-concussion syndrome severity.

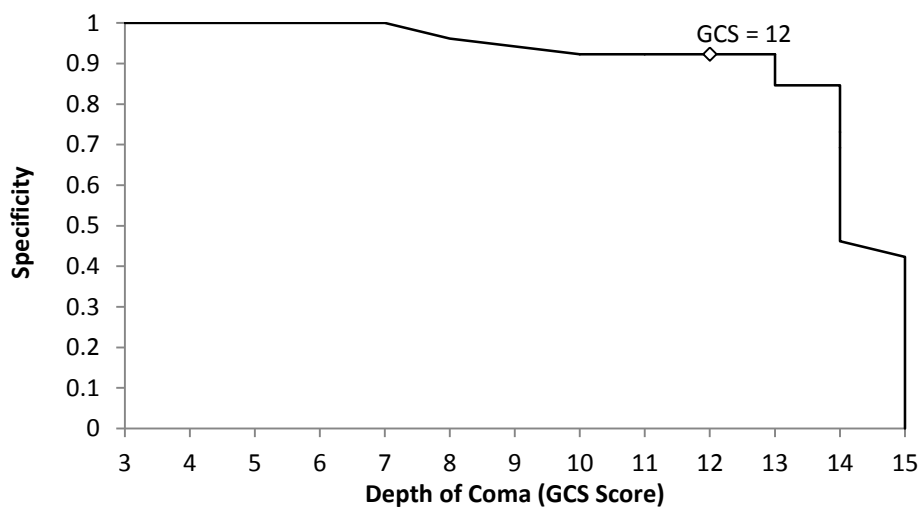


Figure 8.05. The specificity of GCS scores in identifying “Unusually High” post-concussion syndrome severity.

Figures 8.06 and 8.07 below plot the sensitivity and specificity of duration of post-traumatic amnesia (as measured by the WPTA) in identifying “Unusually High” post-concussion syndrome severity (BC-PSI Total Score), the WPTA cut off level of ≥ 8 days is depicted in the plot.

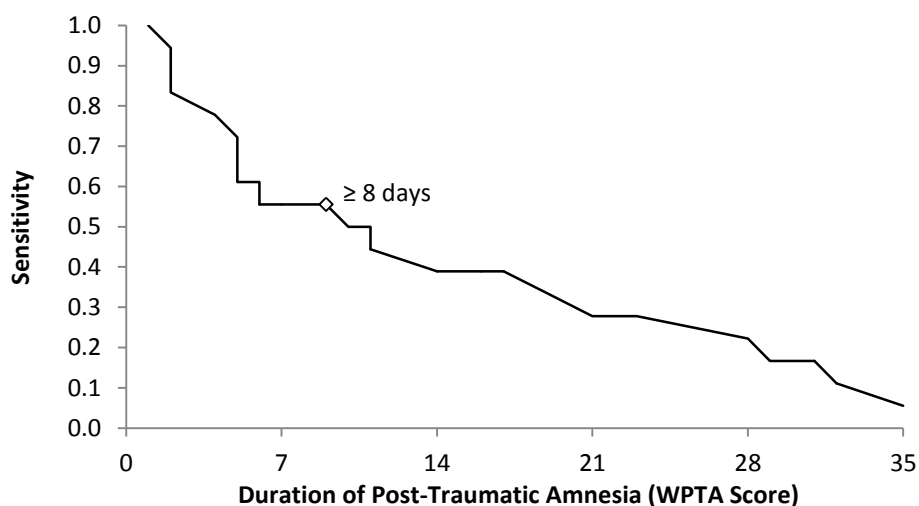


Figure 8.06. The sensitivity of WPTA scores in identifying “Unusually High” post-concussion syndrome severity.

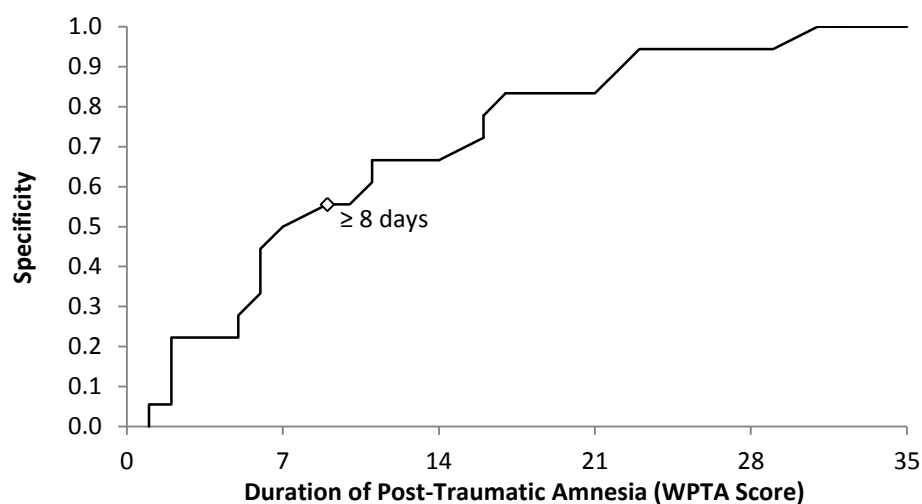


Figure 8.07. The specificity of WPTA scores in identifying “Unusually High” post-concussion syndrome severity.

8.3.5 *Sensitivity and Specificity of Predicting “Extremely High” Post-Concussion Syndrome*

Of the 52 T₁₈₀ participants who sustained a TBI, 16 participants were classified in the “Extremely High” range of post-concussion syndrome severity as measured by the BC-PSI Total Score. The following diagnostic and agreement statistics are for identifying the participants who experienced “Extremely High” post-concussion syndrome severity.

Cohen’s Kappa analysis of using a S100B cut off point of $\geq .97\mu\text{g/L}$ for identifying “Extremely High” BC-PSI Total Scores was significant, and indicated a fair agreement for classification, $\kappa = .271$, $p < .05$, and receiver operating characteristics indicated an AUC of .659. The participants classified as exhibiting “Extremely High” BC-PSI Total Scores had significantly higher S100B levels, $t(17.939) = -1.796$, $p < .01$.

Cohen’s Kappa analysis of using a GCS cut off point of ≤ 12 (“Moderate” depth of coma) for identifying “Extremely High” BC-PSI Total Scores was non-significant, and indicated only a slight agreement for classification, $\kappa = .161$, $p = .200$, and receiver operating characteristics indicated an AUC of .529. The participants classified as exhibiting “Extremely High” BC-PSI Total Scores demonstrated a trend towards lower GCS scores, $t(19.843) = .841$, $p = .059$.

Cohen’s Kappa analysis of using a WPTA cut off point of ≥ 8 days (“Very Severe” post-traumatic amnesia) for identifying “Extremely High” BC-PSI Total Scores was significant, and indicated a fair agreement for classification, $\kappa = .348$, $p < .05$, and receiver operating characteristics indicated an AUC of .668. The participants classified as exhibiting “Extremely High” BC-PSI Total Scores did not have significantly higher WPTA scores, $t(17.471) = 1.713$, $p = .165$.

The Receiver Operator Characteristics for S100B (AUC = .585), GCS (AUC = .579) and WPTA (AUC = .721) in identifying “Extremely High” BC-PSI Total Scores are depicted below in Figure 8.08.

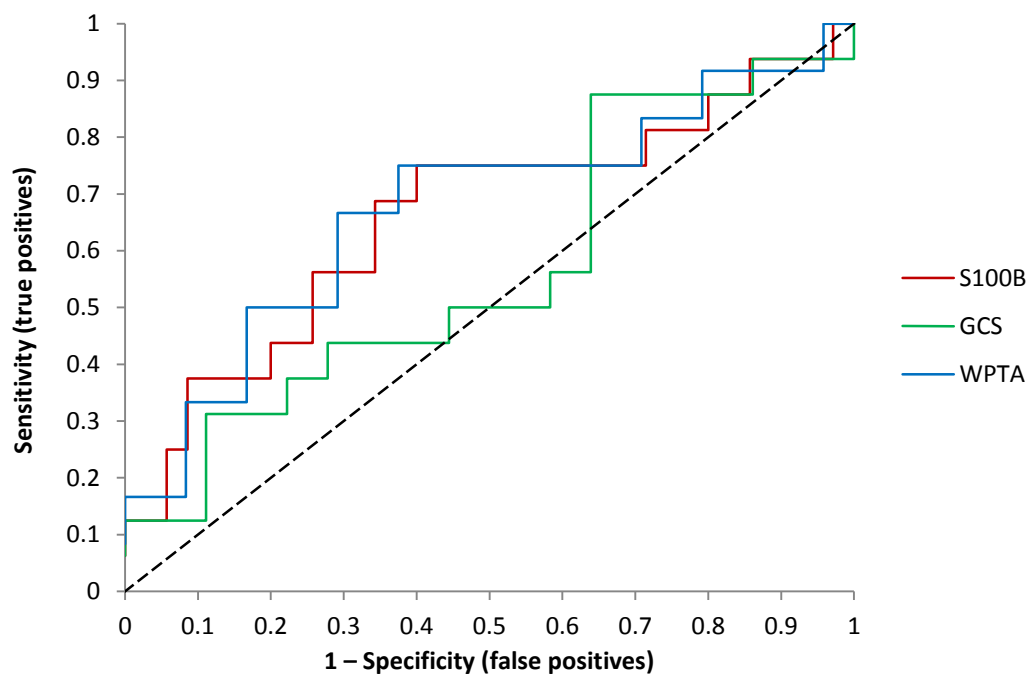


Figure 8.08. Receiver Operating Characteristics of S100B, GCS and WPTA with “Extremely High” BC-PSI Total Scores.

The diagnostic and agreement statistics for S100B using $\geq 0.97\mu\text{g/L}$; GCS using ≤ 12 ; and, WPTA using ≥ 8 days as respective cut offs for identifying “Extremely High” BC-PSI Total Scores are displayed below in Table 8.5.

Table 8.5

Diagnostic and Agreement Statistics for identifying “Extremely High” BC-PSI Scores using S100B, GCS, and WPTA

	S100B		GCS		WPTA	
Sensitivity	.750	(.476 – .927)	.250	(.073 – .524)	.750	(.428 – .945)
Specificity	.571	(.394 – .737)	.889	(.739 – .969)	.625	(.406 – .812)
Efficiency	.627	(.481 – .759)	.692	(.549 – .813)	.667	(.490 – .814)
False Positive Rate	.429	(.263 – .606)	.111	(.031 – .261)	.375	(.188 – .594)
False Negative Rate	.250	(.073 – .524)	.750	(.476 – .927)	.250	(.055 – .572)
Misclassification Rate	.373	(.241 – .536)	.308	(.187 – .535)	.333	(.186 – .386)

Note. 95% confidence intervals are displayed in parentheses. S100B cut off $\geq .97\mu\text{g/L}$, $n = 51$; GCS cut off ≤ 12 , $n = 52$; WPTA cut off ≥ 8 days, $n = 36$.

Figures 8.09 through 8.14 on the following pages illustrate the respective sensitivity and specificity of S100B, GCS and WPTA in identifying “Extremely High” post-concussion syndrome severity (BC-PSI Total Score ≥ 15). The respective cut-offs for each independent variable (established above) are depicted in each plot.

Figures 8.09 and 8.10 below plot the sensitivity and specificity of S100B in identifying “Extremely High” post-concussion syndrome severity (BC-PSI Total Score), the cut off level of .97 μ g/L is depicted in the plot.

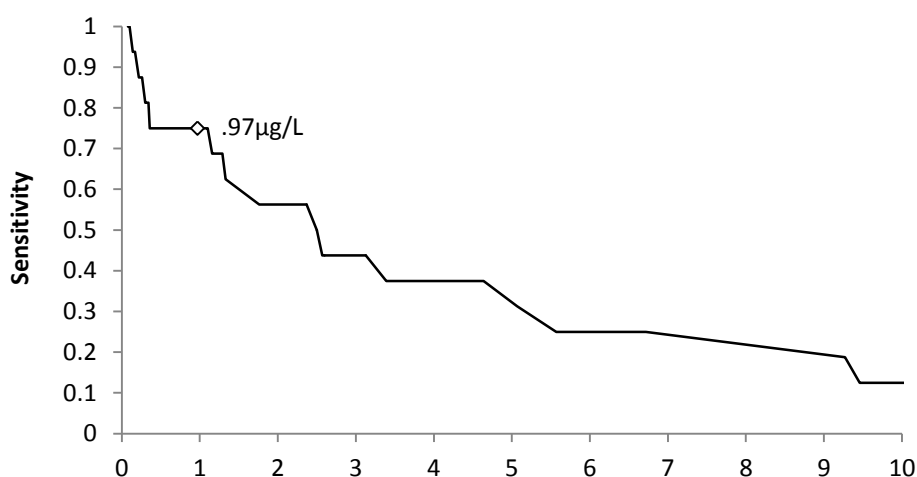


Figure 8.09 The sensitivity of serum S100B levels in identifying “Extremely High” post-concussion syndrome severity.

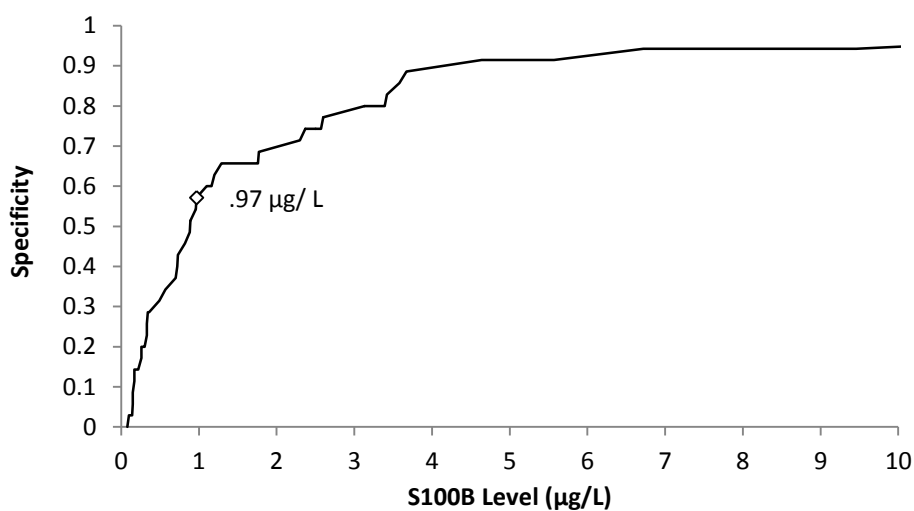


Figure 8.10. The specificity of serum S100B levels in identifying “Extremely High” post-concussion syndrome severity.

Figures 8.11 and 8.12 below plot the sensitivity and specificity of depth of coma (as measured by the GCS) in identifying “Extremely High” post-concussion syndrome severity (BC-PSI Total Score), the GCS cut off score of 12 is depicted in the plot.

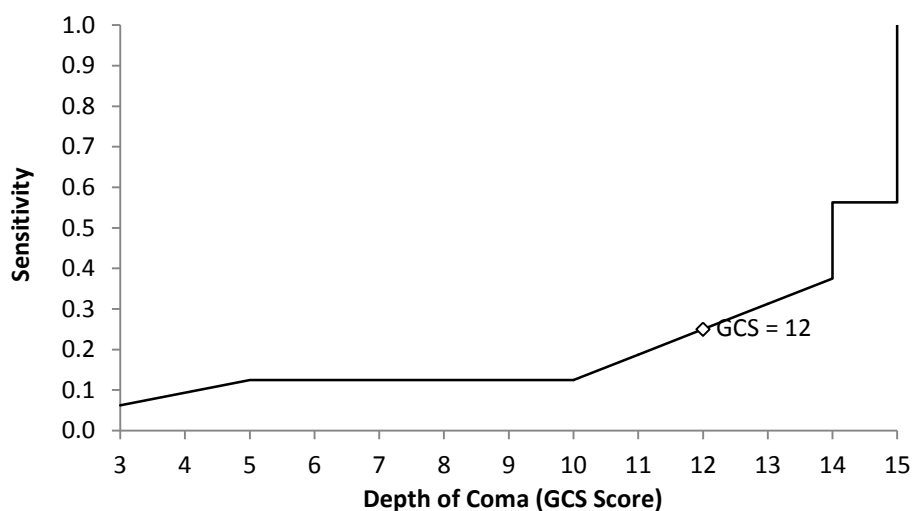


Figure 8.11. The sensitivity of GCS scores in identifying “Extremely High” post-concussion syndrome severity.

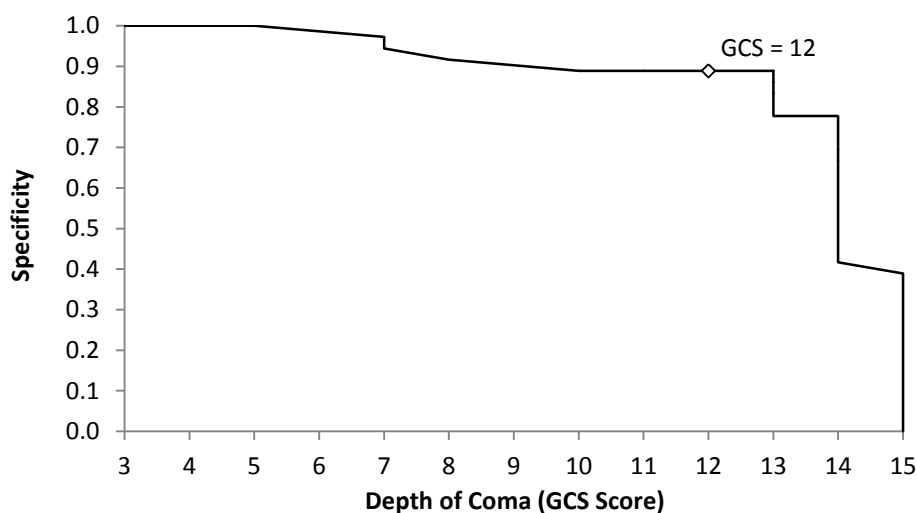


Figure 8.12. The specificity of GCS scores in identifying “Extremely High” post-concussion syndrome severity.

Figures 8.13 and 8.14 below plot the sensitivity and specificity of duration of post-traumatic amnesia (as measured by the WPTA) in identifying “Extremely High” post-concussion syndrome severity (BC-PSI Total Score), the WPTA cut off level of ≥ 8 days is depicted in the plot.

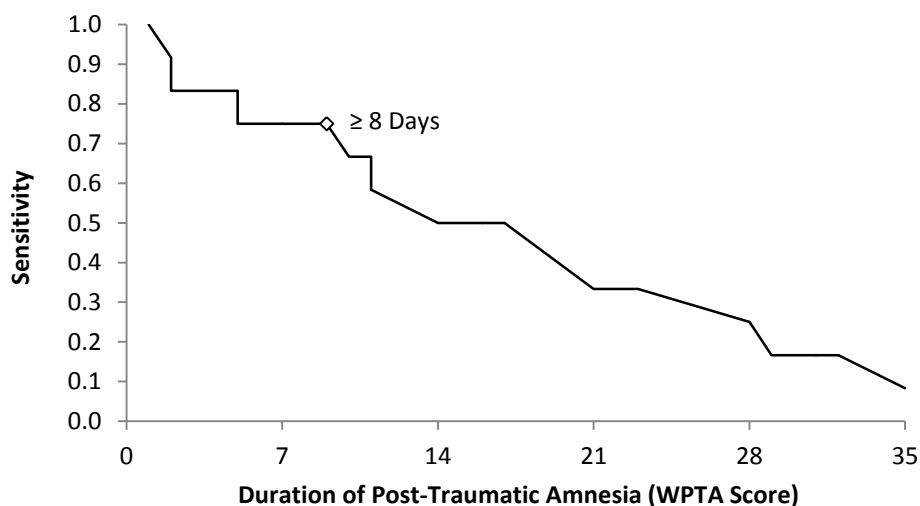


Figure 8.13. The sensitivity of WPTA scores in identifying “Extremely High” post-concussion syndrome severity.

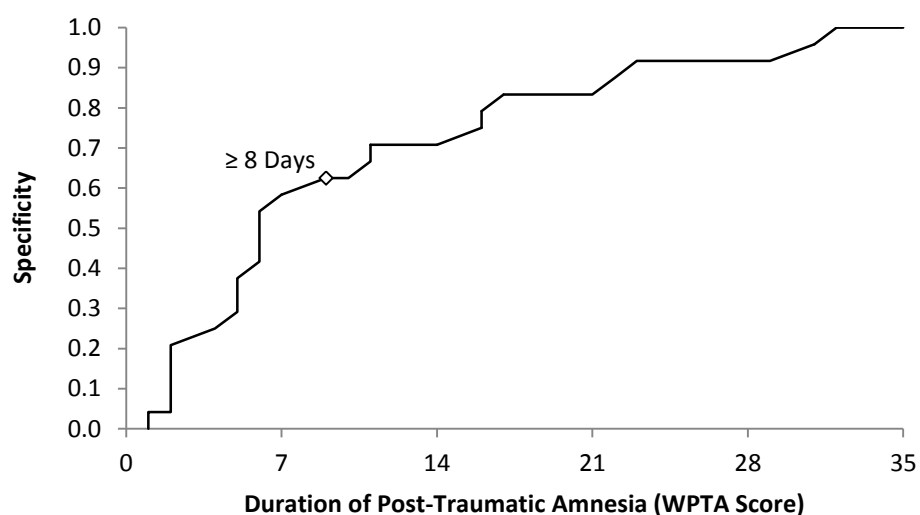


Figure 8.14. The specificity of WPTA scores in identifying “Extremely High” post-concussion syndrome severity.

8.3.6 Nausea/Feeling Sick

Serum S100B levels significantly predicted the severity of the “nausea/feeling sick” BC-PSI item, $R^2 = .096$, $F(1, 50) = 5.201$, $p < .05$. The Jarque-Bera test for normality of this model was significant, $\chi^2(2, N=50) = 344.080$, $p < .001$, and the algorithm for this model is presented in *Equation 8.2*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.2*, $R^2 = .111$, $\Delta R^2 = .015$ $F(2, 50) = 2.995$, $p = .373$, nor did duration of post-traumatic amnesia, $R^2 = .161$, $\Delta R^2 = .013$ $F(2, 34) = 3.059$, $p = .488$.

$$\text{Nausea/Feeling Sick} = (\text{S100B} \times .193) + .320 \quad (\text{Eq. 8.2})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.2* (S100B predicting severity of “Nausea/Feeling Sick”) was significant, $\chi^2(1, N=51) = 144.080$, $p < .001$. Robust regression analysis, however, revealed that S100B remained a significant predictor of WTPA, despite heteroscedasticity of the model ($\beta = .193$, $p < .05$). A post-hoc bivariate Pearson’s correlation revealed a significant strong correlation between severity of nausea, and magnitude of error in the residuals ($.921$, $p < .001$). The residual error across severity of nausea/feeling sick for *Equation 8.2* can be found in Appendix F.

8.3.7 *Feeling Sad*

Serum S100B levels significantly predicted the severity of the “feeling sad” BC-PSI item, $R^2 = .113$, $F(1, 50) = 6.271$, $p < .05$. The Jarque-Bera test for normality of this model was significant, $\chi^2(2, N=51) = 83.073$, $p < .001$, and the algorithm for this model is presented in *Equation 8.3*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.3*, $R^2 = .118$, $\Delta R^2 = .004$ $F(2, 50) = 3.208$, $p = .025$, nor did duration of post-traumatic amnesia, $R^2 = .140$, $\Delta R^2 = .011$ $F(2, 34) = 2.608$, $p = .033$.

$$\text{Feeling Sad} = (\text{S100B} \times .390) + 2.077 \quad (\text{Eq. 8.3})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.3* (S100B predicting severity of “Feeling Sad”) was not significant, $\chi^2(1, N=51) = 0.033$, $p = .856$. A post-hoc bivariate Pearson’s correlation revealed a significant moderate correlation between severity of feeling sad, and magnitude of error in the residuals (.718, $p < .001$). The residual error across severity of feeling sad for *Equation 8.3* can be found depicted in Appendix F.

8.3.8 Poor Concentration

Serum S100B levels significantly predicted the severity of the “poor concentration” BC-PSI item, $R^2 = .117$, $F(1, 50) = 6.503$, $p < .05$. The Jarque-Bera test for normality of this model was significant, $\chi^2(2, N=51) = 47.126$, $p < .001$, and the algorithm for this model is presented in *Equation 8.4*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.4*, $R^2 = .118$, $\Delta R^2 = .001$ $F(2, 50) = 3.202$, $p = .862$, nor did duration of post-traumatic amnesia, $R^2 = .143$, $\Delta R^2 = .048$ $F(2, 34) = 2.669$, $p = .189$.

$$\text{Poor Concentration} = (\text{S100B} \times .427) + 2.035 \quad (\text{Eq. 8.4})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.4* (S100B predicting severity of “Poor Concentration”) was not significant, $\chi^2(1, N=51) = 1.385$, $p = .239$. A post-hoc bivariate Pearson’s correlation revealed a significant moderate correlation between severity of poor concentration, and magnitude of error in the residuals ($.782$, $p < .001$). The residual error across severity of poor concentration for *Equation 8.4* can be found depicted in Appendix F.

8.3.9 Worrying and Dwelling

Serum S100B levels significantly predicted the severity of the “worrying and dwelling” BC-PSI item, $R^2 = .102$, $F(1, 50) = 5.569$, $p < .05$. The Jarque-Bera test for normality of this model was not significant, $\chi^2(2, N=51) = 4.621$, $p=.099$, and the algorithm for this model is presented in *Equation 8.5*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.5*, $R^2 = .118$, $\Delta R^2 = .001$ $F(2, 50) = 3.202$, $p = .862$, nor did duration of post-traumatic amnesia, $R^2 = .146$, $\Delta R^2 = .007$ $F(2, 34) = 2.741$, $p = .600$.

$$\text{Worrying and Dwelling} = (\text{S100B} \times .089) + 1.758 \quad (\text{Eq. 8.5})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.5* (S100B predicting severity of “Worrying and Dwelling”) was not significant, $\chi^2(1, 50) = 1.145$, $p = .285$. A post-hoc bivariate Pearson’s correlation revealed a significant moderate correlation between severity of worrying and dwelling, and magnitude of error in the residuals ($.477$, $p < .001$). The residual error across severity of poor concentration for *Equation 8.5* can be found depicted in Appendix F.

8.4 Quality of Life Results

As per the QOLI protocol (Frisch, 1994), the importance and satisfaction of each QOLI domain were multiplied to produce Weighted Satisfaction Scores for each domain. Table 8.6 displays the participant means and standard deviations for importance, satisfaction, and weighted satisfaction for each of the QOLI domains.

Table 8.6
Participant Means and Standard Deviations for the Domains of the QOLI

Domain	Importance	Satisfaction	Weighted Satisfaction
Health	1.65 (0.52)	0.65 (2.03)	1.12 (3.65)
Self-Esteem	1.44 (0.64)	1.06 (1.80)	1.62 (3.16)
Goals and Values	1.48 (0.58)	1.00 (1.90)	1.62 (3.21)
Money	1.08 (0.62)	0.75 (1.87)	0.58 (2.52)
Work	1.12 (0.51)	0.60 (1.98)	0.46 (2.62)
Play	1.46 (0.58)	1.21 (1.64)	1.87 (2.77)
Learning	1.12 (0.65)	1.29 (1.60)	1.54 (2.30)
Creativity	1.08 (0.71)	1.02 (1.59)	1.25 (2.21)
Helping	1.29 (0.64)	1.10 (1.62)	1.69 (2.45)
Love	1.50 (0.67)	1.04 (1.96)	1.83 (3.48)
Friends	1.50 (0.67)	2.04 (1.43)	3.48 (2.58)
Children	1.25 (0.79)	2.00 (1.40)	2.81 (2.85)
Relatives	1.25 (0.65)	1.88 (1.38)	2.87 (2.35)
Home	1.60 (0.50)	2.02 (1.38)	3.27 (2.50)
Neighbourhood	1.13 (0.56)	1.81 (1.46)	2.37 (2.21)
Community	1.15 (0.64)	1.38 (1.39)	1.88 (2.13)

Note. $N = 52$.

QOLI Total Scores are calculated by dividing the total sum of Weighted Satisfaction Score by the number of domains that the respondent cited as being either “important” or “extremely important” (i.e., not “not important”). Table 8.7 displays frequencies for Total QOLI scores at each classification level.

Table 8.7
Classification Frequencies for QOLI Total Scores

	Very Low	Low	Average	High
Total Score	14 (26.92%)	5 (9.62%)	20 (38.46%)	13 (25.00%)

Note. $N = 52$.

8.4.1 Correlations with Domains of Quality of Life

Two-tailed Pearson's correlations were conducted between serum S100B levels, depth of coma (GCS), duration of post-traumatic amnesia (WPTA), and domains of quality of life as assessed by the QOLI. Correlation analyses were also conducted for S100B, GCS, and WPTA with QOLI Total Scores, and Thomas et. al's (2012) QOLI Factor Scores (Factor 1: Self-Functioning & Activity; Factor 2: Self-Actualisation; and, Factor 3: Family & Environment)

As displayed in Table 8.8, S100B was significantly moderately correlated with QOLI Total Score, Thomas et al.'s Factor 2 (Self-Actualisation), goals and values, learning, creativity, helping, and love; GCS was significantly moderately correlated with helping, and friends; and WPTA was significantly moderately correlated with QOLI Total Score, Thomas et. al's Factor 3 (Family & Environment), love, friends, and home.

Table 8.8
Correlations between S100B, GCS, and WPTA with the Total Score, Thomas et al. Factors, and Domains of the QOLI

Factor/Domain	S100B (n=51)	GCS (n=52)	WPTA (n=36)
QOLI Total Score	-.282*	-.150	-.344
Thomas et al. Factor 1	-.209	.067	-.242
Thomas et al. Factor 2	-.303*	.198	-.256
Thomas et al. Factor 3	-.253	.150	-.363*
Health	-.125	-.062	-.102
Self-Esteem	-.241	.077	-.290
Goals and Values	-.287*	.008	-.263
Money	-.158	.163	-.226
Work	-.038	.257	-.190
Play	-.088	.075	-.116
Learning	-.303*	.102	-.191
Creativity	-.301*	.164	-.315
Helping	-.321*	.308*	-.237
Love	-.323*	.255	-.474**
Friends	-.013	.274*	-.346*
Children	-.196	-.130	-.052
Relatives	-.078	-.140	-.014
Home	-.143	.109	-.398*
Neighbourhood	-.213	.019	-.011
Community	-.016	.110	-.034

Note. * $p < .05$ ** $p < .01$, two-tailed; $N = 52$.

8.4.2 QOLI Total Score

Serum S100B levels significantly predicted participants' QOLI Total Score, $R^2 = .079$, $F(1, 50) = 4.220$, $p < .05$. The Jarque-Bera test for normality of this model was not significant, $\chi^2(2, N=51) = 1.822$, $p = .402$, and the algorithm for this model is presented in *Equation 8.6*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.6*, $R^2 = .086$, $\Delta R^2 = .006$ $F(2, 50) = 2.251$, $p = .116$, nor did duration of post-traumatic amnesia, $R^2 = .127$, $\Delta R^2 = .065$ $F(2, 34) = 2.326$, $p = .114$.

$$\text{QOLI Total Score} = (\text{S100B} \times -1.914) + 35.005 \quad (\text{Eq. 8.6})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.6* was not significant, $\chi^2(1, N=51) = .040$, $p = .842$. A post-hoc bivariate Pearson's correlation revealed there was no significant correlation between QOLI Total Scores and magnitude of error in the residuals ($-.250$, $p=.077$). The residual error across QOLI Total Scores (expressed as *T*-Scores for clinical interpretation) for *Equation 8.6* can be found depicted in Appendix F.

8.4.3 Sensitivity and Specificity of Predicting "Low" Quality of Life

Of the 52 T_{180} participants who sustained a TBI, 19 participants were classified in the "Low" ($T\text{-Score} = 37\text{-}42$; $n = 5$) or "Very Low" ($T\text{-Score} = 0\text{-}36$; $n = 14$) range of quality of life as measured by the QOLI Total Score. The following diagnostic and agreement statistics are for identifying the participants who experienced at least "Low" ($T\text{-Score} \leq 42$; $n = 19$) overall quality of life.

Using $\geq .97\mu\text{g/L}$ as a S100B cut-off point for identifying “Low” QOLI Total scores, Cohen’s Kappa analysis of this cut off point was non-significant and indicated a fair agreement, $\kappa = .259$, $p = .055$, and receiver operating characteristics indicated an AUC of .633. The participants classified as exhibiting “Low” QOLI Total Scores had significantly higher S100B levels, $t(24.041) = -1.501$, $p < .05$.

Cohen’s Kappa analysis using ≤ 12 as a GCS cut-off point for identifying “Low” QOLI Total scores was non-significant, and indicated only a slight agreement for classification, $\kappa = .102$, $p = .390$, and receiver operating characteristics indicated an AUC of .524. The participants classified as exhibiting “Low” QOLI Total Scores had significantly lower GCS scores, $t(23.947) = -1.036$, $p < .05$.

Cohen’s Kappa analysis using a WPTA cut off point of ≥ 8 days (“Very Severe” post-traumatic amnesia) for identifying “Low” QOLI Total Scores was non-significant, and indicated only a slight agreement for classification, $\kappa = .167$, $p = .298$, and receiver operating characteristics indicated an AUC of .640. The participants classified as exhibiting “Low” QOLI Total Scores did not have significantly higher WPTA scores, $t(19.691) = -1.390$, $p = .147$.

The Receiver Operator Characteristics for S100B (AUC = .633), GCS (AUC = .524) and WPTA (AUC = .640) in identifying “Low” QOLI Total Scores are depicted below in Figure 8.15.

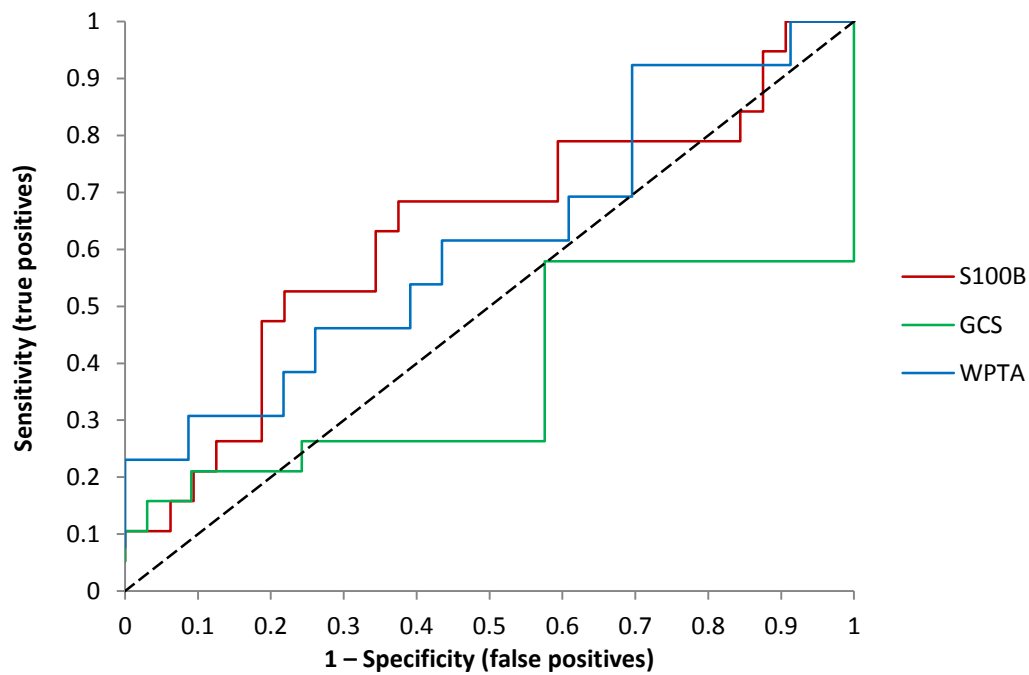


Figure 8.15. Receiver Operating Characteristics of S100B, GCS and WPTA with “Low” QOLI Total Scores.

The diagnostic and agreement statistics for S100B using $\geq .97\mu\text{g/L}$; GCS using ≤ 12 ; and, WPTA using ≥ 8 days as respective cut offs for identifying “Low” QOLI Total Scores are displayed below in Table 8.9

Table 8.9

Diagnostic and Agreement Statistics for identifying “Low” QOLI Scores using S100B, GCS, and WPTA

	S100B		GCS		WPTA	
Sensitivity	.684	(.434 – .874)	.211	(.061 – .456)	.615	(.316 – .861)
Specificity	.594	(.406 – .763)	.879	(.718 – .966)	.565	(.345 – .768)
Efficiency	.627	(.481 – .759)	.635	(.490 – .764)	.583	(.408 – .745)
False Positive Rate	.406	(.237 – .594)	.121	(.034 – .282)	.435	(.232 – .655)
False Negative Rate	.316	(.126 – .566)	.789	(.544 – .939)	.385	(.139 – .684)
Misclassification Rate	.373	(.241 – .428)	.365	(.236 – .606)	.417	(.255 – .514)

Note. 95% confidence intervals are displayed in parentheses. S100B cut off $\geq .97\mu\text{g/L}$, $n = 51$; GCS cut off ≤ 12 , $n = 52$; WPTA cut off ≥ 8 days, $n = 36$.

Figures 8.16 through 8.21 on the following pages illustrate the respective sensitivity and specificity of S100B, GCS and WPTA in identifying “Low” quality of life (QOLI Total T -Score ≤ 42). The respective cut-offs for each independent variable (established above) are depicted in each plot.

Figures 8.16 and 8.17 below plot the sensitivity and specificity of S100B in identifying “Low” quality of life (QOLI Total Score), the cut off level of .97 $\mu\text{g/L}$ is depicted in the plot.

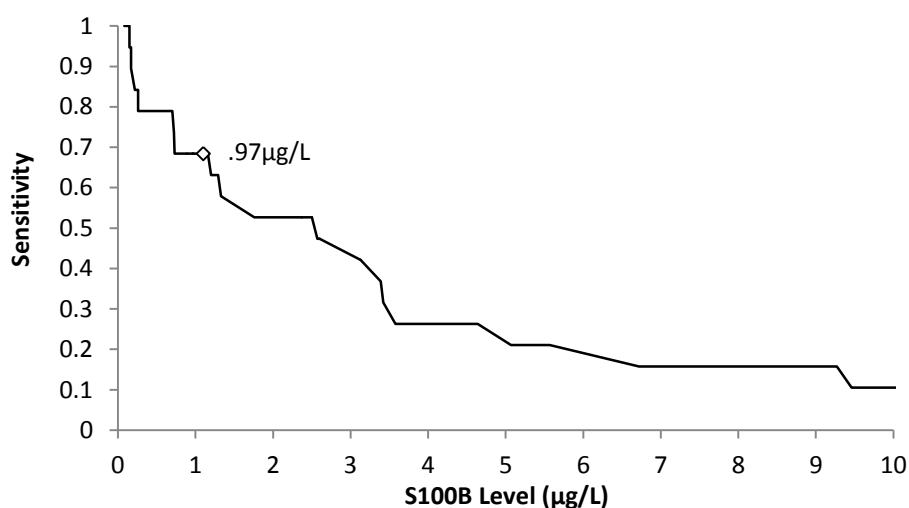


Figure 8.16. The sensitivity of serum S100B levels in identifying “Low” quality of life.

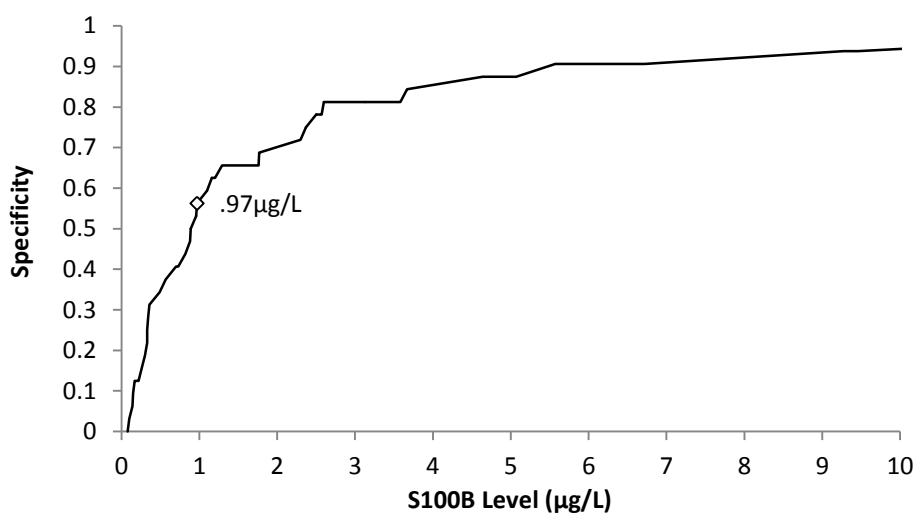


Figure 8.17. The specificity of serum S100B levels in identifying “Low” quality of life.

Figures 8.18 and 8.19 below plot the sensitivity and specificity of depth of coma (as measured by the GCS) in identifying “Low” quality of life (QOLI Total Score), the cut off level of 12 is depicted in the plot.

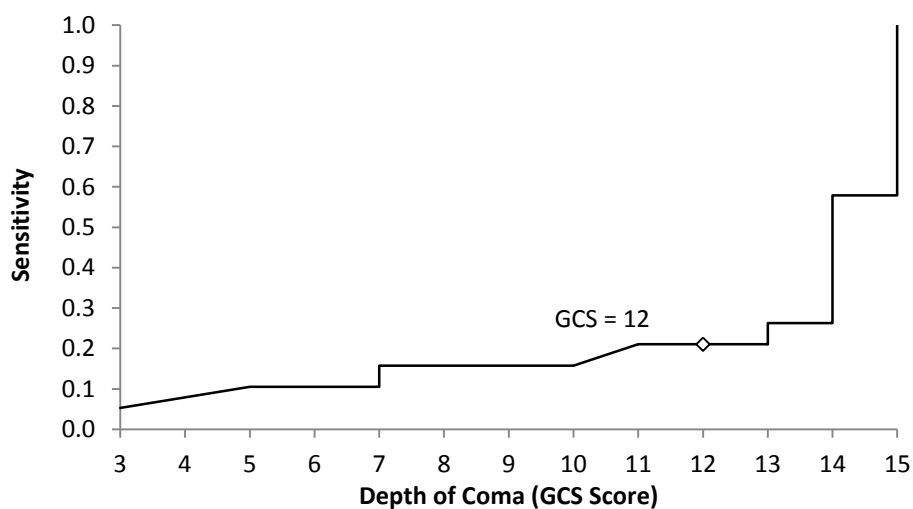


Figure 8.18. The sensitivity of GCS scores in identifying “Low” quality of life.

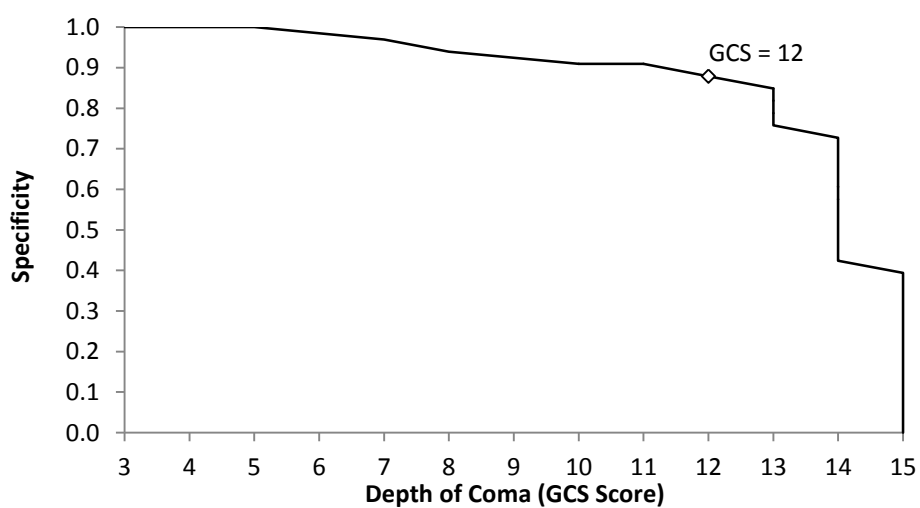


Figure 8.19. The specificity of GCS scores in identifying “Low” quality of life.

Figures 8.20 and 8.21 below plot the sensitivity and specificity of duration of post-traumatic amnesia (as measured by the WPTA) in identifying “Low” quality of life (QOLI Total Score), the cut off level of ≥ 8 days is depicted in the plot.

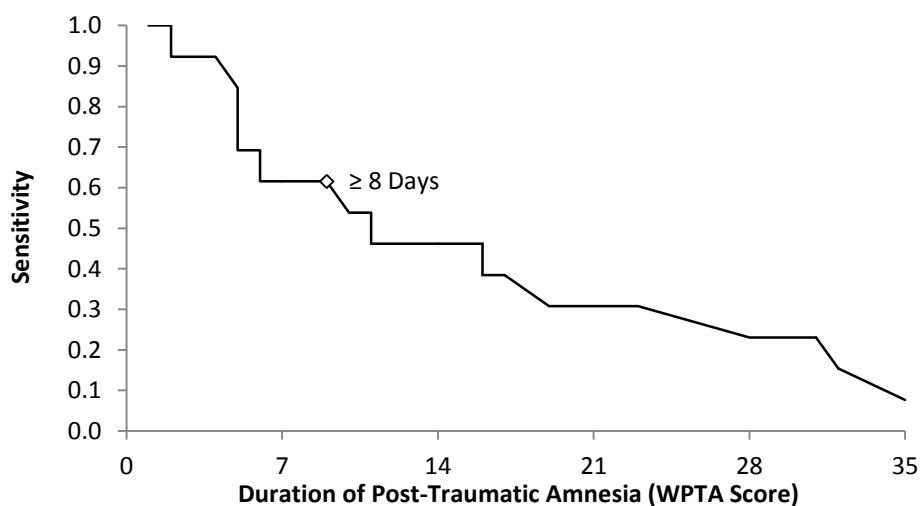


Figure 8.20. The sensitivity of WPTA scores in identifying “Low” quality of life.

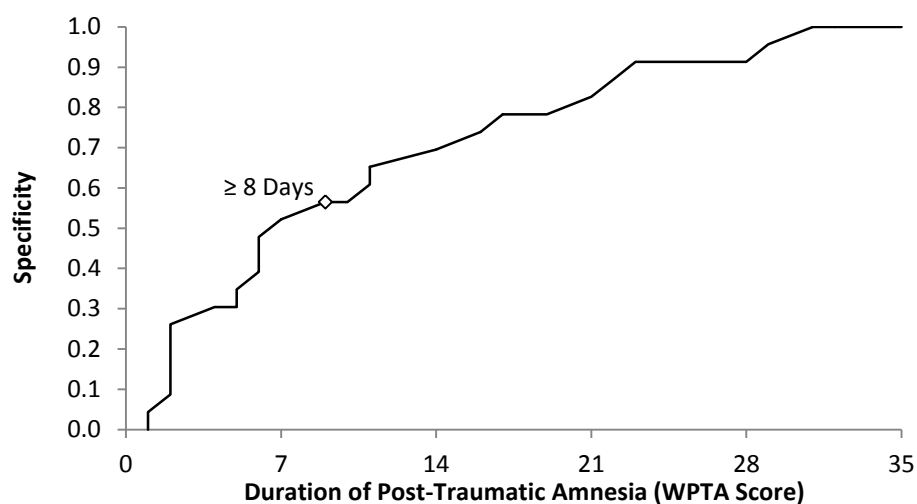


Figure 8.21. The specificity of WPTA scores in identifying “Low” quality of life.

8.4.4 Sensitivity and Specificity of Predicting “Very Low” Quality of Life

Of the 52 T₁₈₀ participants who sustained a TBI, 14 participants were classified in the “Very Low” range of quality of life as measured by the QOLI ($T\text{-Score} \leq 36$). The following diagnostic and agreement statistics are for identifying the participants who experienced “Very Low” quality of life.

Using .97µg/L as a S100B cut off point for identifying “Very Low” QOLI Total scores, Cohen’s Kappa analysis of this cut off point was non-significant and indicated only a slight agreement for classification, $\kappa = .145$, $p = .242$. Receiver operating characteristics indicated an AUC of .583. The participants classified as exhibiting “Very Low” QOLI Total Scores had significantly higher S100B levels, $t(14.947) = -1.375$, $p < .01$.

Cohen’s Kappa analysis using ≤ 12 as a GCS cut-off point for identifying “Very Low” QOLI Total scores was non-significant, and indicated only a slight agreement for classification, $\kappa = .102$, $p = .390$. Receiver operating characteristics indicated an AUC of .477. The participants classified as exhibiting “Very Low” QOLI Total Scores had significantly lower GCS scores, $t(14.853) = 1.302$, $p < .001$.

Cohen’s Kappa analysis using a WPTA cut off point of ≥ 8 days (“Very Severe” post-traumatic amnesia) for identifying “Very Low” QOLI Total Scores was non-significant, and indicated only a slight agreement for classification, $\kappa = .222$, $p = .109$. Receiver operating characteristics indicated an AUC of .723. The participants classified as exhibiting “Very Low” QOLI Total Scores had significantly higher WPTA scores, $t(8.647) = -1.798$, $p < .05$.

The Receiver Operator Characteristics for S100B (AUC = .633), GCS (AUC = .524) and WPTA (AUC = .640) in identifying “Very Low” QOLI Total Scores are depicted below in Figure 8.22.

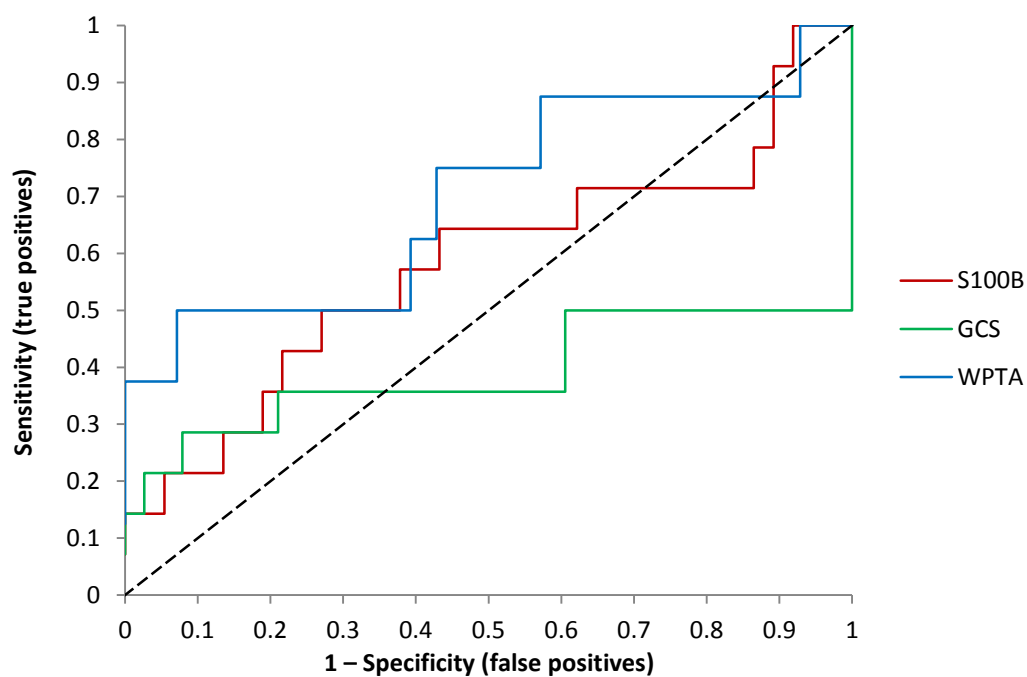


Figure 8.22. Receiver Operating Characteristics of S100B, GCS and WPTA with “Very Low” QOLI Total Scores.

The diagnostic and agreement statistics for S100B using $\geq .97\mu\text{g/L}$; GCS using ≤ 12 ; and, WPTA using ≥ 8 days as respective cut offs for identifying “Very Low” QOLI Total Scores are displayed below in Table 8.10.

Table 8.10

Diagnostic and Agreement Statistics for identifying “Very Low” QOLI Scores using S100B, GCS, and WPTA

	S100B		GCS		WPTA	
Sensitivity	.643	(.351 – .872)	.286	(.084 – .581)	.750	(.349 – .968)
Specificity	.541	(.369 – .705)	.895	(.752 – .971)	.571	(.372 – .755)
Efficiency	.569	(.422 – .707)	.731	(.590 – .844)	.611	(.435 – .769)
False Positive Rate	.459	(.295 – .631)	.105	(.029 – .248)	.429	(.245 – .628)
False Negative Rate	.357	(.128 – .649)	.714	(.419 – .916)	.250	(.032 – .651)
Misclassification Rate	.431	(.293 – .421)	.269	(.156 – .481)	.389	(.231 – .349)

Note. 95% confidence intervals are displayed in parentheses. S100B cut off $\geq .97\mu\text{g/L}$, $n = 51$; GCS cut off ≤ 12 , $n = 52$; WPTA cut off ≥ 8 days, $n = 36$.

Figures 8.23 through 8.28 on the following pages illustrate the respective sensitivity and specificity of S100B, GCS and WPTA in identifying “Very Low” quality of life (QOLI Total T -Score ≤ 36). The respective cut-offs for each independent variable (established above) are depicted in each plot.

Figures 8.23 and 8.24 below plot the sensitivity and specificity of S100B in identifying “Very Low” quality of life (QOLI Total Score), the cut off level of .97 μ g/L is depicted in the plot.

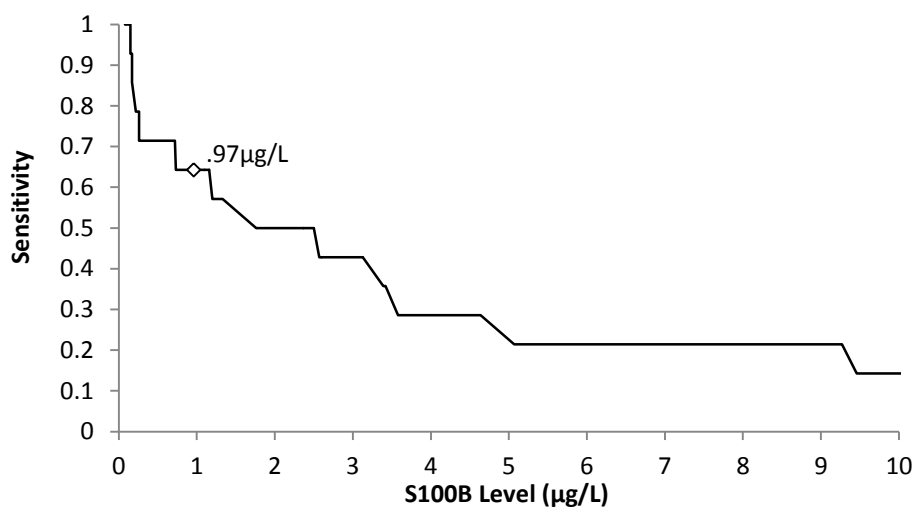


Figure 8.23. The sensitivity of serum S100B levels in identifying “Very Low” quality of life.

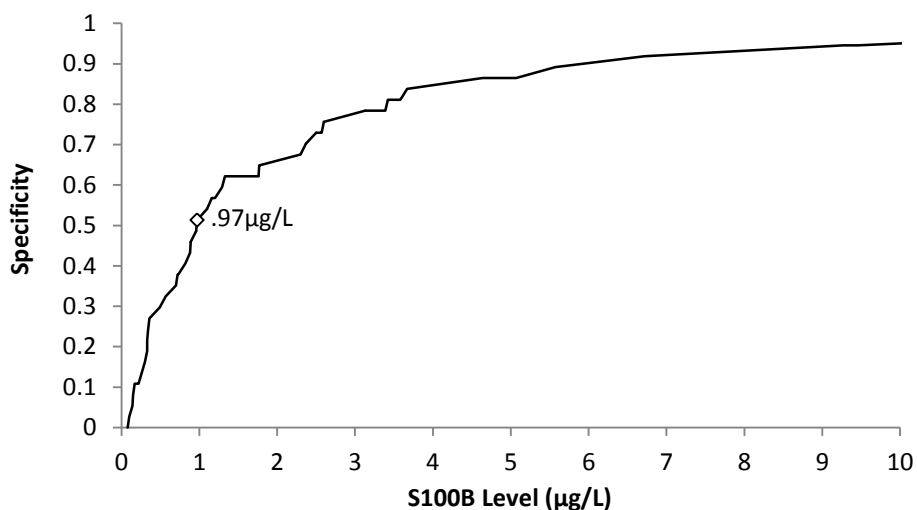


Figure 8.24. The specificity of serum S100B levels in identifying “Very Low” quality of life.

Figures 8.25 and 8.26 below plot the sensitivity and specificity of depth of coma (as measured by the GCS) in identifying “Very Low” quality of life (QOLI Total Score), the cut off level of GCS = 12 is depicted in the plot.

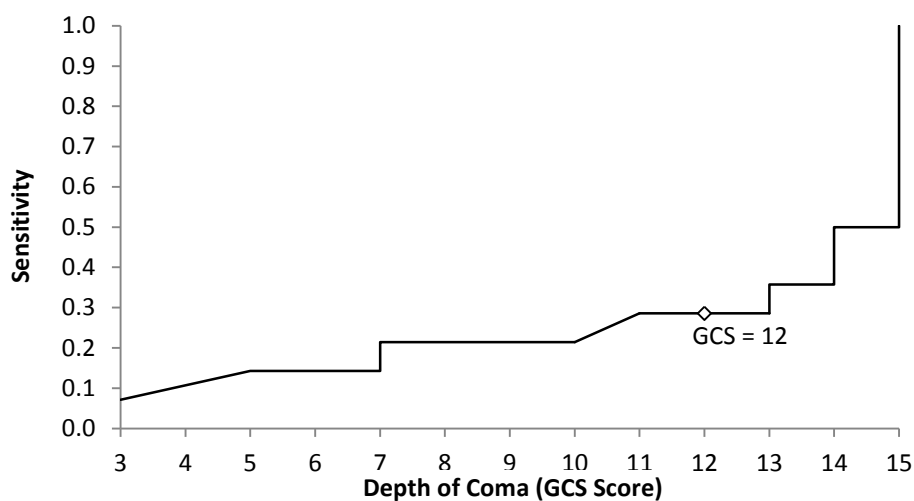


Figure 8.25. The sensitivity of GCS scores in identifying “Very Low” quality of life.

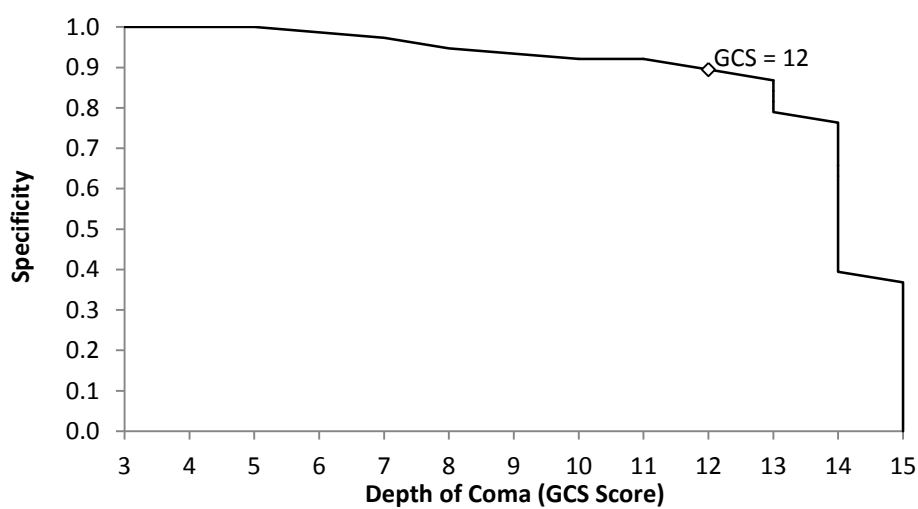


Figure 8.26. The specificity of GCS scores in identifying “Very Low” quality of life.

Figures 8.27 and 8.28 below plot the sensitivity and specificity of duration of post-traumatic amnesia (as measured by the WPTA) in identifying “Very Low” quality of life (QOLI Total Score), the cut off level of ≥ 8 days is depicted in the plot.

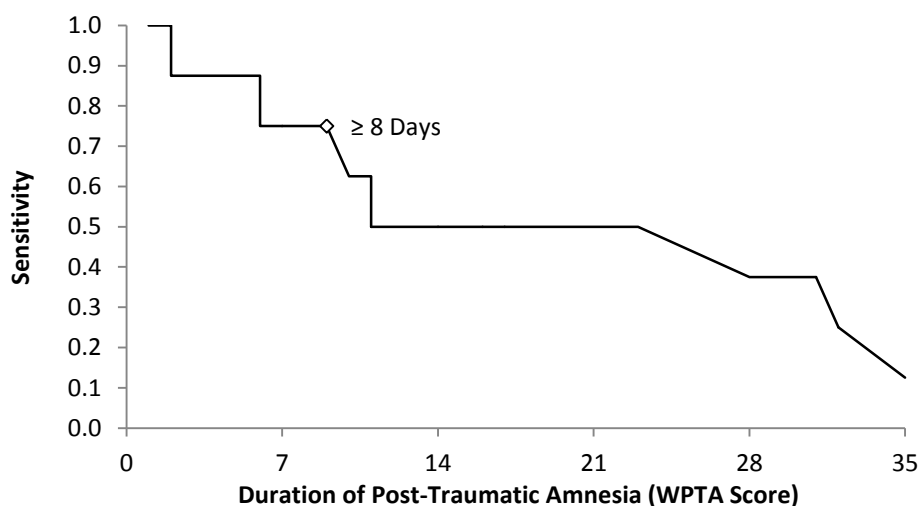


Figure 8.27. The sensitivity of WPTA scores in identifying “Very Low” quality of life.

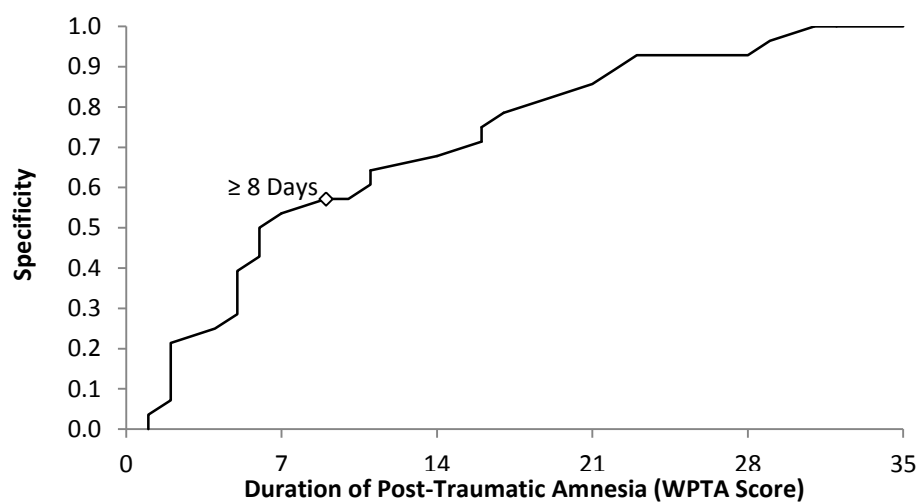


Figure 8.28. The specificity of WPTA scores in identifying “Very Low” quality of life.

8.4.5 Thomas et. al.'s Factor 2: Self-Actualisation

Serum S100B levels significantly predicted scores on the Thomas et. al.'s (2012) "Self-Actualisation" QOLI Factor, $R^2 = .092$, $F(1, 50) = 4.965$, $p < .05$. The Jarque-Bera test for normality of this model was not significant, $\chi^2(2, N=51) = .917$, $p = .632$, and the algorithm for this model is presented in *Equation 8.7*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.7*, $R^2 = .107$, $\Delta R^2 = .015$ $F(2, 50) = 2.885$, $p = .066$, nor did duration of post-traumatic amnesia, $R^2 = .111$, $\Delta R^2 = .015$ $F(2, 34) = 1.992$, $p = .153$.

$$\text{Self-Actualisation} = (\text{S100B} \times -.147) + 1.948 \quad (\text{Eq. 8.7})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.7* was not significant, $\chi^2(1, N=51) = .031$, $p = .861$. A post-hoc bivariate Pearson's correlation revealed there was no significant correlation between Self-Actualisation scores and magnitude of error in the residuals ($.159$, $p = .264$). The residual error across Self-Actualisation for *Equation 8.7* can be found depicted in Appendix F.

8.4.6 Goals and Values

Serum S100B levels significantly predicted the weighted satisfaction of the "Goals and Values" QOLI domain, $R^2 = .082$, $F(1, 50) = 4.403$, $p < .05$. The Jarque-Bera test for normality of this model was not significant, $\chi^2(2, N=51) = 2.640$, $p = .276$, and the algorithm for this model is presented in *Equation 8.8*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.8*, $R^2 = .087$, $\Delta R^2 = .005$ $F(2, 50) = 2.287$, $p = .113$, nor did duration of post-traumatic amnesia, $R^2 = .119$, $\Delta R^2 = .016$ $F(2, 34) = 2.164$, $p = .131$.

$$\text{Goals and Values} = (\text{S100B} \times -.228) + 2.187 \quad (\text{Eq. 8.8})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.8* was not significant, $\chi^2(1, N=51) = 1.239, p = .266$. A post-hoc bivariate Pearson's correlation revealed a significant weak correlation between weighted satisfaction of goals and values, and magnitude of error in the residuals (.335, $p < .05$). The residual error across weighted satisfaction of goals and values for *Equation 8.8* can be found depicted in Appendix F.

8.4.7 Learning

Serum S100B levels significantly predicted the weighted satisfaction of the "Learning" QOLI domain, $R^2 = .092, F(1, 50) = 4.969, p < .05$. The Jarque-Bera test for normality of this model was significant, $\chi^2(2, N=51) = 19.615, p < .001$, and the algorithm for this model is presented in *Equation 8.9*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.9*, $R^2 = .092, \Delta R^2 = .000, F(2, 50) = 2.439, p = .098$, nor did duration of post-traumatic amnesia, $R^2 = .106, \Delta R^2 = .001, F(2, 34) = 1.905, p = .165$.

$$\text{Learning} = (\text{S100B} \times -.167) + 1.904 \quad (\text{Eq. 8.9})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.9* was not significant, $\chi^2(1, N=51) = .005, p = .947$. A post-hoc bivariate Pearson's correlation revealed there was no significant correlation between weighted satisfaction of "Learning", and magnitude of error in the residuals (.164, $p = .250$). The residual error across weighted satisfaction of learning for *Equation 8.9* can be found depicted in Appendix F.

8.4.8 Creativity

Serum S100B levels significantly predicted the weighted satisfaction of the “Creativity” QOLI domain, $R^2 = .091$, $F(1, 50) = 4.890$, $p < .05$. The Jarque-Bera test for normality of this model was significant, $\chi^2(2, N=51) = 61.200$, $p < .001$, and the algorithm for this model is presented in *Equation 8.10*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.10*, $R^2 = .100$, $\Delta R^2 = .009$ $F(2, 50) = 2.659$, $p = .080$, nor did duration of post-traumatic amnesia, $R^2 = .106$, $\Delta R^2 = .030$ $F(2, 34) = 3.016$, $p = .063$.

$$\text{Creativity} = (\text{S100B} \times -.166) + 1.685 \quad (\text{Eq. 8.10})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.10* was not significant, $\chi^2(1, N=51) = .001$, $p = .982$. A post-hoc bivariate Pearson’s correlation revealed there was no significant correlation between weighted satisfaction of “Creativity,” and magnitude of error in the residuals ($.043$, $p=.764$). The residual error across weighted satisfaction of creativity for *Equation 8.10* can be found depicted in Appendix F.

8.4.9 Helping

Serum S100B levels significantly predicted the weighted satisfaction of the “Helping” QOLI domain, $R^2 = .103$, $F(1, 50) = 5.614$, $p < .05$. The Jarque-Bera test for normality of this model was not significant $\chi^2(2, N=51) = .519$, $p=.771$, and the algorithm for this model is presented in *Equation 8.11*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.11* $R^2 = .162$, $\Delta R^2 = .058$ $F(2, 50) = 4.606$, $p = .074$, nor did duration of post-traumatic amnesia $R^2 = .109$, $\Delta R^2 = .013$ $F(2, 34) = 1.960$, $p = .502$.

$$\text{Helping} = (\text{S100B} \times -.196) + 2.218 \quad (\text{Eq. 8.11})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.11* was not significant, $\chi^2(1, N=51) = .080, p = .777$. A post-hoc bivariate Pearson's correlation revealed there was no significant correlation between weighted satisfaction of "Helping," and magnitude of error in the residuals (.042, $p=.772$). The residual error across weighted satisfaction of helping for *Equation 8.11* can be found depicted in Appendix F.

8.4.10 Love

Serum S100B levels significantly predicted the weighted satisfaction of the "Love" QOLI domain, $R^2 = .103, F(1, 50) = 5.690, p < .05$. The Jarque-Bera test for normality of this model was significant $\chi^2(2, N=51) = 21.964, p < .001$, and the algorithm for this model is presented in *Equation 8.12*. Depth of coma did not significantly add to the variance accounted for by *Equation 8.12* $R^2 = .146, \Delta R^2 = .042 F(2, 50) = 4.106, p = .131$ – however, duration of post-traumatic amnesia significantly added to the variance accounted for by this equation $R^2 = .273, \Delta R^2 = .144 F(2, 34) = 6.007, p < .05$. The Jarque-Bera test for normality of this improved model was not significant $\chi^2(2, N=35) = .098, p=.952$, and the algorithm for this model is presented in *Equation 8.13*.

$$\text{Love} = (\text{S100B} \times -.276) + 2.651 \quad (\text{Eq. 8.12})$$

$$\text{Love} = (\text{S100B} \times -.134) \times (\text{WPTA} \times 155) + 4.071 \quad (\text{Eq. 8.13})$$

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.12* was not significant, $\chi^2(1, N=51) = 1.355, p = .244$. A post-hoc bivariate Pearson's correlation revealed there was no significant correlation between weighted satisfaction of "Love," and magnitude of error in the residuals ($-.200, p=.160$).

A Breusch-Pagan test for heteroscedasticity conducted on *Equation 8.13* was not significant, $\chi^2(1, N=35) = .124, p = .725$. A post-hoc bivariate Pearson's correlation revealed there was no significant correlation between weighted satisfaction of "Love," and magnitude of error in the residuals ($-.066, p=.706$). The residual error across weighted satisfaction of love for *Equation 8.12* and *Equation 8.13* can be found depicted in Appendix F.

8.5 Discussion

This study investigated the prognostic utility of serum S100B concentrations with reference to the severity of symptoms of post-concussion syndrome (PCS) and quality of life experienced by TBI patients 180 days following injury. More specifically, the present study quantified the accuracy that S100B holds in predicting the presence and severity of PCS symptoms and factors relating to quality of life that can be chronic and enduring for patients that have suffered a TBI.

8.5.1 S100B and Post-Concussion Syndrome

S100B has been shown to be associated with symptoms of PCS at two weeks to eight months post-injury (Savola & Hillborn, 2003), and three months post-injury (Ingebrigsten et al., 2000). However, no such significant associations were found in the studies of Bazarian et al. (2006), and Stålnacke et al. (2005). In alignment with the proposed relationship, however, the current study hypothesised that serum

concentrations of S100B would be significantly correlated with the presence and severity of PCS symptoms, and as a result, the clinical classification of PCS could be predicted by patients' S100B values.

These hypotheses were supported by the data in that S100B was significantly moderately correlated with PCS symptoms of feeling sad, nausea and feeling sick, poor concentration, and worrying and dwelling on symptoms. Further, regression analyses illustrated that neither GCS nor WPTA accounted for any significant additional variance in the outcome of these symptoms. This result supports the results of Ingebrigsten et al. (2000) who reported that mild TBI patients who tested positive for S100B, exhibited increased frequencies of somatic and cognitive symptoms three months post injury, rather than symptoms of psychological origin. Although the primary complaints identified in the present study were poor sleep, memory problems, and fatigue, with over 25% of the participants rating these symptoms as "Moderate-Severe," it can be argued that S100B's ability to account for variance in the less endorsed symptoms gives it clinical utility towards the more variable nuances of post TBI sequelae.

Further to the above, the results of the present study illustrated that S100B was able to categorically discriminate between those that reported "Extremely High" range post-concussion symptomatology, as assessed by the BC-PCSI, and those that did not. This result extends the implications drawn by Savola and Hillbom's (2003) study of 172 mild TBI patients. The researchers interviewed patients using a modified Rivermead Post-Concussion Symptoms Questionnaire (RPQ; King et al., 1995), and a follow-up interview at eight months to ensure chronicity of the endorsed symptoms. Savola and Hillbom concluded that S100B can be used as a specific

predictor of PCS, and that their results indicate an organic aetiology for the PCS symptoms that are associated with elevated serum concentrations of S100B.

While the area under the curve for S100B correctly classifying “Extremely High” PCS symptomatology was only poor to fair, and comparable to that of the WPTA, it should be noted that those patients had significantly higher serum S100B levels, but not significantly longer durations of post-traumatic amnesia. Further, S100B and WPTA held identically moderate properties of sensitivity indicating equivalent clinical risk of false-positive classification, whereas WPTA held stronger specificity indicating less risk of false-negative classification. As such, clinicians considering both serum S100B levels and WPTA scores in a combined prognostic rubric would benefit from increased accuracy in identifying those patients at risk of enduring chronic severe PCS symptomatology.

8.5.2 *S100B and Quality of Life*

As discussed in the preceding literature review, S100B has been shown to be associated with poorer quality of life (Woertgen et al., 2002), prolonged return to work (Stranjalis et al., 2004; Metting et al., 2012), poorer somatic health (Stålnacke et al., 2013), and symptoms related to stress disorders (Sojka et al., 2006). Consistent with the associated literature, this study hypothesised that serum concentrations of S100B would be significantly inversely correlated with domains of quality of life following TBI, and further, that the clinical classification of poorer quality of life could be predicted by patients’ serum concentrations of S100B.

These hypotheses were somewhat supported by the data of the present study, in that overall quality of life, as assessed by the QOLI Total Score, could be predicted by serum S100B levels, and neither GCS scores nor WPTA duration

significantly accounted for any additional variance. While there was no statistical issue with residual error across the continuum of quality of life, it should be noted that S100B only accounted for eight percent of the variance in this measure. Further, despite significantly higher S100B levels being found for the patients who experienced “Low” and “Very Low” quality of life, S100B was inefficient in correctly classifying these patients. Similar results were found for GCS and WPTA indicating that no acute independent variable was statistically efficient in identifying those that experienced poorer overall quality of life, and those that did not.

In light of the above, S100B concentrations were shown to be significantly correlated with various domains of quality of life – namely: self-actualisation; goals and values; learning; creativity; helping; and love. Neither GCS nor WPTA significantly accounted for any additional variance in these domains except for WPTA to the domain of love. Interestingly, the domains of quality of life that S100B was correlated with were also the domains that the participants rated as being least satisfied with.

These domain specific findings are consistent with the longitudinal research by Oddy, Coughlan, and Tyerman (1985) who observed that most TBI patients experienced pronounced difficulty in successfully establishing romantic relationships after their injury. Further, Stålnacke et al.’s (2005) study of S100B and life satisfaction one year post TBI found that patients with higher levels of S100B had poorer life satisfaction, largely attributable to higher levels of disability. The researchers added that the domains where TBI patients were “very dissatisfied” were those of finances, leisure, sex life, and relationships. The present study extends this area of the literature by evidencing a potential biological constituent and predictor of such outcomes. Further, this study is the first to have utilised the QOLI, a well

established measure with validation within TBI populations, as a dependent variable in the examination of the prognostic utility of serum S100B.

8.5.3 *Conclusions*

Following the acute treatment of management of TBI, the longer term chronicity of post-concussion symptoms and their effect on daily life and functioning is difficult to predict. For most patients, these symptoms typically abate across the first six months following their injury – however, for some patients, these impacts are enduring. Findings of the present study indicated that serum concentrations of S100B are significantly correlated with various symptoms of PCS and domains of quality of life following TBI. Although S100B in isolation offers insufficient prognostic accuracy for these clinical outcomes, the results of the present study hold implications for future research into biomarkers for TBI. The study contributes to the associated literature by incorporating extensively validated measures of outcome. Future research into S100B, or any other potential biomarker for TBI, would benefit from the translational implications of using such measures.

Chapter 9

General Discussion

9.1 *Discussion of Findings*

The aim of the present thesis was to investigate the prognostic utility for serum S100B following TBI, with specific emphasis on neuropsychological functioning as the definable outcomes. As such, this thesis aimed to elucidate the ability for S100B to make prognoses for duration of post-traumatic amnesia, severity of cognitive impairment, presence of symptoms of post-concussion syndrome, and deficits in domains that are associated with quality of life.

Study One: T₀ investigated the utility of serum S100B with reference to the diagnosis and management of TBI immediately following injury. More specifically, the first study elucidated the diagnostic, categorical, and operational utility of a single collection of serum S100B by identifying statistical relationships between serum levels of S100B and the current acute measures for classifying TBI. Consistent with hypotheses, serum S100B concentrations were found to be significantly correlated with GCS and WPTA scores. In short, the higher the level of S100B, the deeper the coma following injury and the longer the post-traumatic amnesia experienced by the patient. Categorically, S100B was able to discriminate between mild, moderate, and severe depths of coma as measured by the GCS, and also, which patients would endure “Very Severe” post-traumatic amnesia (greater than one week following injury) as measured by the WPTA. The results of the first study contribute to the empirical research concerned with validation of S100B in the medical management of TBI, and extend the associated literature by investigating the biomarker’s utility with a clinical variable that is currently used in the treatment and management of TBI – namely the WPTA.

Study Two: T₆₀ investigated the prognostic utility of serum S100B with reference to cognitive impairment experienced by TBI patients 60 days post injury. More specifically, the second study aimed to quantify the accuracy that S100B holds in predicting the magnitude of ipsative impairment experienced by TBI patients, across a variety of standardised measures of cognitive ability. It was hypothesised that serum S100B levels would be significantly correlated with the magnitude of ipsative impairment on each of the measures of cognitive ability. Contrary to hypotheses, however, S100B was not significantly correlated with ipsative impairment on any of the cognitive subtests utilised in this study. Further, regression analyses for cognitive impairment illustrated that S100B made no significant contributions to any prognostic models. Despite the absence of clinical significance, all correlations between serum concentrations of S100B and ipsative cognitive impairment were found to be in the hypothesised direction.

Although results of the second study indicated that S100B concentrations were not significantly correlated with the magnitude of any ipsative cognitive impairment, it should be acknowledged that GCS and WPTA scores could not prognosticate impairment either. The results illustrate that cognitive impairment is remarkably difficult to predict from information available in the acute setting. Further, from a translational perspective, the second study illustrates that any accurate prognoses relating to cognitive impairment following TBI require acute neuropsychological assessment and consultation, and that prognoses based on biological factors alone while in an acute setting remains elusive, if not illusive. By incorporating an ipsative impairment model of post injury cognitive functioning as a dependent variable, the second study extends the associated TBI biomarker literature.

Study Three: T₁₈₀ investigated the prognostic utility of serum S100B concentrations with reference to the severity of symptoms of post-concussion syndrome (PCS) and quality of life experienced by TBI patients 180 days following injury. More specifically, the third study aimed to quantify the accuracy that S100B holds in predicting the presence and severity of PCS symptoms and factors relating to quality of life that can be chronic and enduring for patients that have suffered a TBI. As hypothesised, S100B was significantly moderately correlated with PCS symptoms of feeling sad, nausea and feeling sick, poor concentration, and worrying and dwelling on symptoms. Further, S100B levels were able to categorically discriminate between those that reported “Extremely High” range post-concussion symptomatology, as assessed by the BC-PCSI, and those that did not – however, the area under the curve for correctly classifying “Extremely High” PCS symptomatology was only poor to fair.

With reference to quality of life, S100B concentrations were shown to be significantly correlated with various domains of quality of life as measured by the QOLI – namely: self-actualisation; goals and values; learning; creativity; helping; and love. Neither GCS nor WPTA significantly accounted for any additional variance in these domains except for WPTA to the domain of love. Results of the third study also showed that overall quality of life, as assessed by the QOLI Total Score, could be predicted by serum S100B levels, and neither GCS scores nor WPTA duration significantly accounted for any additional variance. It should be noted that S100B only accounted for eight percent of the variance in this measure, and despite significantly higher S100B levels being found for the patients who experienced “Low” and “Very Low” quality of life, S100B was inefficient in correctly classifying these patients. Similar results were found for GCS and WPTA indicating that no

acute independent variable was statistically efficient in identifying those that experienced poorer overall quality of life and those that did not. Overall, results of the third study indicated that serum concentrations of S100B are significantly correlated with various symptoms of PCS and domains of quality of life following TBI. Although S100B in isolation offers insufficient prognostic accuracy for these clinical outcomes, the results of the present study hold directions for future research into S100B and other biomarkers for TBI, and translational implications for “bench top to bedside” experimental methodology.

Taken together, results of the present thesis possess some clinical implications for the use of serum S100B following TBI. First, by ascertaining a patient’s S100B level and depth of coma on arrival at hospital, clinicians making an unfavourable post-traumatic amnesia prognosis can do so with confidence informed by concise convergent validation. Unfortunately, however, S100B cannot be used to prognose cognitive impairment. Clinical examination of indices of brain injury severity, medical imaging, comprehensive history taking, and neuropsychological assessment remain the best practice for attaining an accurate prognosis for cognitive functioning following TBI. Further, despite correlations suggesting a relationship between S100B levels and the longer term chronicity of post-concussion symptoms and their effect on daily life and functioning, S100B in isolation offers insufficient prognostic accuracy for these clinical outcomes. This thesis contributes to the associated literature by incorporating extensively validated measures of functional outcome that are of direct relevance to the survivors of TBI. Future research into S100B, or any other potential biomarker for TBI, would benefit from the translational implications of using such measures and methodologies.

9.2 *Limitations and Considerations*

Despite the growth in S100B research, most studies are difficult to compare because the methodology and statistical reporting between studies is often not uniform (Papa et al., 2013). Timing of blood collection is perhaps the least uniformed constituent in the experimental designs of the associated literature, and Ohrt-Nissen et al. (2011) suggest that blood samples should be collected at a consistent and specific time point after the injury. S100B levels used in this thesis were ascertained by analysing an aliquot of serum from each patient's earliest collected sample. Although the mean collection time in this study is comparable to most studies, the methodology used in this thesis meant that post-injury collection times were not standardised. No corrective manipulations were made to serum S100B levels used in this study.

Beyond standardising a consistent post-injury collection time, the optimal time for sampling S100B in serum has been widely discussed in the associated literature. Typically, serum S100B levels are at their maximum shortly after injury before progressively dropping in concentration across the next one to two days (Egea-Guerrero et al., 2013). The present study utilised patients' first available blood collection, and in Thelin et al.'s (2013) metaanalysis, the authors consider initial samples to be the most important in predicting outcome. However, other studies have markedly different methodologies. For example, some studies have collected serum across several time-points and used the mean of these collections as the independent variable (Ucar et al., 2004; Pelinka et al., 2004; Naemi et al., 2006), whereas others have used the peak level obtained across the collections (Raabe et al., 1999; Woertgen et al., 1999; Townend et al., 2002).

Being that this study was granted ethical approval on the basis that no additional procedures would be necessary for the patient, and that analyses would be performed on aliquots from routinely collected samples, standardised sampling and serial sampling was not possible. Consistent with the assertions of Papa et al. (2013), future studies of biomarkers will require more rigorous and uniform research methodology. As such, efforts should be made in future research towards identifying the optimal sampling time for determining prognoses.

A further limitation of this study is that no controls were made for potential extracranial sources of S100B, and therefore, some participants' serum concentrations may have been elevated attributable to sources beyond the nature of their TBI pathology. S100B's expression in adipocytes, chondrocytes, and melanocytes are argued to conflict and confuse the data in TBI studies (Walder et al., 2013; Yokobori et al., 2013), and impact on its prognostic utility as a biomarker for TBI (Ohrt-Nissen et al., 2011). Given the aetiologies of injury in the present thesis, it can be assumed that many of the participants would have suffered extracerebral injuries, comorbid to their TBI. These comorbid traumas, particularly to regions of the body with higher adiposity, have been shown to result in significantly higher S100B levels than head injuries in isolation (Ohrt-Nissen et al., 2011). However, this result was not evident in the research conducted by Pham et al. (2010), and it is further argued that any elevations in S100B concentrations from extracranial sources are clinically inconsequential to a confirmed TBI, as their contribution to levels are significantly lower than the contribution made by TBI (Lange et al., 2010). Nevertheless, the present thesis did not collect data pertaining to extracranial trauma, nor investigate the significance of participants' comorbid injuries to their serum S100B concentrations. Future research may need to consider these investigations.

Lastly, although the initial participant sample of 127 is one of the largest in S100B/TBI research – regrettably, the number of participants meeting criteria at each of the three time points ($T_0 N = 79$; $T_{60} N = 50$; $T_{180} N = 52$) impacts the power of the analyses and the conclusions that can be drawn from the findings. This research is currently being continued by another team of researchers, and it is anticipated that the increased participant pool from their studies will strengthen the findings of the current thesis.

9.3 *Future Directions for TBI Biomarkers*

Beyond S100B, other serum proteins are emerging in the associated literature as potential biomarkers for TBI. One such protein is glial fibrillary acidic protein (GFAP). GFAP is expressed in the central nervous system, and its breakdown products (GFAP-BDP) are released following parenchymal brain injury. Recent exploratory studies have shown GFAP and GFAP-BDP to reliably distinguish the presence and severity of CT imaging results (Okonkwo et al., 2013), and to discriminate between TBI patients and healthy controls (Diaz-Arrastia et al., 2013). Other proteomic biomarker candidates that have emerged in the recent TBI literature include ubiquitin c-terminal hydrolase (UCHL1; Berger et al., 2012), alpha-II spectrin breakdown products (α II-SBDP; Weiss et al., 2009), heart fatty acid binding protein (Walder et al., 2013), interleukin 8 (IL-8; Stein et al., 2012), and interleukin 10 (IL-10; Soares et al., 2012). Given the methodology of the present thesis, it is possible to analyse the stored serum samples for concentrations of any of the above proteins in order to directly compare and contrast their diagnostic and prognostic utility for neuropsychological outcomes.

Jeter et al. (2013) argue that, based on the biomarkers tested to date, it is unlikely that a single biomarker will have sufficiently robust utility to serve as a standalone diagnostic test for TBI, or to predict which patients are most likely to experience unfavourable outcomes. Sharma and Laskowitz (2012) add that the direction of future research may be to enhance sensitivity and specificity by combining a panel of biomarkers, similar to troponin and creatine kinase isoenzymes M and B for diagnosis in myocardial infarction (Guzy, 1977). Papa et al (2013) add that, due to the heterogeneity of TBI pathologies, devising a panel of biomarkers may prove to be useful in distinguishing the various pathoanatomic processes that occur during injury. Devising such a panel is the intended direction for future research to come from the present thesis.

Further to the specific biomarker being studied, the statistical modelling techniques for future prognostic research deserve methodological consideration. One such emerging technique is group-based trajectory modelling (GBTM; Niyonkuru et al., 2013). In the GBTM approach, clusters of individuals following similar biomarker trajectories over time are identified and stratified. The methodology assumes that the clinical population is composed of distinct groups, defined by their biomarker trajectories, and these groups are therefore likely to have heterogeneous outcomes. Further, the prognostic utility of S100B has been shown to be enhanced by the algorithmic inclusion of clinical variables, such as the GCS (Lo et al., 2010; Wolf et al., 2013). Future research may benefit from investigating the inclusion of other clinical variables such as imaging classification (Marhall et al., 1992) or acute neuropsychological examination results in order to investigate any mediation effects on the accuracy of functional prognoses.

9.4 *Summary*

This thesis aimed to quantify the prognostic utility of S100B for neuropsychological outcomes including duration of post-traumatic amnesia, severity of cognitive impairment, presence of symptoms of post-concussion syndrome, and deficits in domains that are associated with quality of life. The clinical implications from this research indicate that serum S100B can be used by clinicians to make prognoses for a patient's post-traumatic amnesia, however it cannot be used to prognose cognitive impairment. Further, despite correlations suggesting a relationship between S100B levels and the longer term chronicity of post-concussion symptoms and their effect on daily life and functioning, S100B in isolation offers insufficient prognostic accuracy for these clinical outcomes.

This thesis possesses several limitations. Firstly, although the mean collection time in this study is comparable to most studies, the methodology used in this thesis meant that post-injury collection times were not standardised and serial sampling was not possible. Further, no controls were made for potential extracranial sources of S100B – however, the impact of the contribution made by these sources is equivocal in the associated literature. Lastly, the number of participants meeting criteria at each of the three time points impacts the power of the analyses and the conclusions that can be drawn from the findings.

Future TBI prognostic research would benefit from investigating proteomic candidates as they emerge, and considering a biomarker panel in order to distinguish various pathoanatomic processes that occur during injury. Further, from a translational perspective, future TBI biomarker research would benefit from utilising neuropsychological outcomes in their experimental design in order to make direct implications to the unique symptoms that are experienced by survivors of TBI.

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